NORTHERN BOTTLENOSE WHALES IN CANADA: THE STORY OF EXPLOITATION, CONSERVATION, AND RECOVERY

by

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Dedication

To my daughter
Ione Mae Feyrer Edwards

“**Three Things to Remember**

As long as you’re dancing, you can
break the rules.

Sometimes breaking the rules is just
extending the rules.

Sometimes there are no rules.”

– Mary Oliver

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Abstract

Many populations of cetaceans are still recovering from the impacts of whaling and face ongoing, and sometimes increasing, human stressors. However, due to their slow reproductive life history, monitoring trends and responses to potential threats requires long-term datasets. Understanding the status of cryptic species, like beaked whales, is further challenged by their remote habitat, low density and deep diving ability, restricting opportunities for data collection. Northern bottlenose whales (NBW, *Hyperoodon ampullatus*) were previously whaled and, although the Scotian Shelf population is currently recognized as Endangered under the *Species at Risk Act* (*SARA*), their population structure as well as their status in Canada are unclear. The goal of my thesis was to reassess evidence for NBW population structure, life history and threats using a diversity of methods to integrate long-term datasets and address questions broadly relevant to the conservation of beaked whales. I began with a systematic review and metanalysis of the literature on cetacean population structure with particular consideration of studies of odontocetes. I applied genetics to distinguish evolutionarily significant population structure and historical demography of NBW in Canada and stable isotopes to estimate the period of maternal care. Using photoidentification I reviewed the stability of marks and quantified the rate of anthropogenic scarring. I modelled the change in cumulative human impacts over 30 years to evaluate concurrent trends in estimates of NBW abundance and spatial conservation measures. I found that, despite having almost the lowest genetic diversity of any cetacean, Scotian Shelf NBW were distinct from those in other areas, supporting their management as a Designatable Unit under *SARA*. However, their recovery from whaling has likely been impacted by their low reproductive potential, and despite few reports, ongoing threats of entanglement. Encouragingly, long term trends indicate that the reduction of human activities on the Scotian Shelf has contributed to the population's recovery. My results demonstrate how the life history and past exploitation of NBW have left them vulnerable to ongoing impacts, and suggest that protecting important habitat can be an effective conservation tool for beaked whales.

List of Abbreviations

AIC – Akaike Information Criterion
AOI – Area of Interest
Bp – Base pairs
CBD – Convention on Biological Diversity
CHI – Cumulative human impacts
CNLOPB – Canada-Newfoundland and Labrador Offshore Petroleum Board
CNSOPB – Canada-Nova Scotia Offshore Petroleum Board
COSEWIC – Committee on the Status of Endangered Wildlife in Canada
DFO – Department of Fisheries and Oceans
DIP – Demographically independent populations
DMSO – Dimethyl-sulphoxide
DU – Designated Unit
EEZ – Exclusive economic zone
ESU – Evolutionarily significant units
FJ – Female-Juveniles
GLG – Growth layer group
GLM – Generalized linear regression models
HPDI – Highest Posterior Density Intervals
He – Expected heterozygosity
Ho – Observed heterozygosity
ID – Individual
IUCN – International Union for the Conservation of Nature
LGM – Last glacial maximum
MCMC – Markov chain Monte Carlo
MMFAS – Military mid-frequency active sonar
MMM – Males-mature males
MPA – Marine protected area
mtDNA – Mitochondrial DNA
NBW – Northern bottlenose whale
N_c – Census population size
N_e – Effective population size
PCR – Polymerase chain reaction
PBR – Potential biological removal
RMSE – Root mean square error
SARA – Species at Risk Act
SST – Sea surface temperature
TMRCA – Time to most recent common ancestor
WAIC – Watanabe-Akaike information criteria

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Without this support my research would not have been possible.

Chapter 1 Introduction

The aim of my thesis is to advance our understanding of northern bottlenose whale (NBW, *Hyperoodon ampullatus*) population structure, life history and threats to improve the scientific management and conservation of beaked whales in Canada and around the world. In this chapter I provide an overview of my specific research questions, the significance of my work, my research objectives and key findings. The conservation and biology of NBW has been an active area of research out of Dalhousie University for over thirty years and yet important questions regarding their population dynamics, social structure, ecology and life history remain. Given the paucity of data and technical challenges in studying cryptic cetaceans like beaked whales, long-term field studies provide a critically important foundation for understanding the fundamental biology of wildlife, as well as monitoring the status and potential threats faced by small populations (Hooker et al., 2019; Lindenmayer et al., 2012; Shaffer, 1981; Soulé, 1987). My research aimed to integrate contemporary and historical datasets, using a diversity of methods to address questions broadly relevant for not only the conservation and management of the Scotian Shelf population NBW, but other populations of beaked whales around the world as well. I explored questions related to the definition of evolutionarily significant units for management and the boundaries between populations, the consequences of low genetic diversity for small populations, the implications of maternal care for life history and reproductive potential, the challenges of identifying cryptic threats and quantifying their impact, the efficacy of marine protected areas and the evaluation of trends in abundance for long lived species.

The recognition of at least three new species of beaked whales over the last 20 years (Dalebout et al., 2003, 2014; Yamada et al., 2019) and the recent discovery of a fourth putative species (MacKinnon, 2021), indicates we still have much to learn about the members of this family of cetaceans. Inspired by the need to conserve rapidly declining global biodiversity, Canada ratified the United Nations Convention on Biological Diversity (CBD) in 1992, and in the era that followed there has been considerable investment in research and conservation of species at risk. Unfortunately the local, ecological and commercial extinctions of marine species have also been ongoing at an alarming rate (Ceballos et al., 2017; McCauley et al., 2015); with an estimated ~90% still awaiting description (Mora et al., 2011) and only a fraction of known species having enough data to assess their status (Pimm et al., 2014). Recognizing our efforts to identify and conserve the biodiversity of marine species is a race against time, we must learn lessons where we can. Outstanding questions on the biology, life history, status and efficacy of conservation measures should not go unanswered because organisms are rare or hard to study. Scientific management, and the survival of some evolutionarily distinct species and populations, may depend on data driven status assessments, scientific advances and our perseverance. Given the urgency of unprecedented future climate change and ongoing marine defaunation, the significance of fundamental research on data-poor species like beaked whales cannot be understated.

1.1 The Ziphiidae

Globally the family Ziphiidae, is composed of at least 23 recognized extant species, with a similar number of extinct members, which first appeared in the fossil record beginning sometime in the early Miocene (17-5 mya) (Bianucci et al., 2016; Committee on Taxonomy, 2021; Kawatani & Kohno, 2021). Dalebout et al.'s (2008) genetic phylogeny characterized the radiation of the diversity of modern beaked whales as being largely driven by sexual selection, suggesting the distinct tusks and bony maxillary crests of mature males in each species function as secondary sexual characteristics or ‘antlers on the inside’(Gol’din, 2014). More recently Bianucci et al. (2016) reassessed fossil evidence and proposed the radiation of two convergent Ziphiid clades occurred independently in the Southern Ocean and the North Atlantic, likely in response to selective pressures for deep diving and suction feeding on soft bodied teuthivores (Lindberg & Pyenson, 2007). The six genera currently alive today descending from crown Ziphiidae include the cosmopolitan *Ziphius cavirostris* and 15 *Mesoplodon* spp., the three *Berardius* spp., with two restricted to the North Pacific and one in the Southern Ocean, the monotypic *Indopacetus pacificus* and *Tasmacetus shepherdi*, whose range is poorly understood but assumed to be limited to the Southern Oceans, and two species of *Hyperoodon,* one in the North Atlantic and one in the Southern Ocean. (Kawatani & Kohno, 2021). The anti-tropical distribution of the two species in the genus *Hyperoodon* is likely the result of vicariant equatorial warming, as implicated in the evolution of other high-latitude cetaceans (Davies, 1963). Long isolated from their congener, the Southern bottlenose (*Hyperoodon planifrons*), the body size of *H. ampullatus* (average length ranging from 7-9m) is larger than *Mesoplodon* spp. (average 5m), similar to *Z. cavirostris* (average 8m), but smaller than *Berardius* spp. (average 9-12m), who also inhabit colder high latitude waters (Ellis & Mead, 2017; MacLeod, 2006).

1.2 Northern Bottlenose Whales – A 'Model Organism'?

Northern bottlenose whales, "Baleine à bec commune", are one of the most well-known beaked whale species in the diverse family Ziphiidae, found only in the northern North Atlantic (Figure 1.1). Our understanding of their biology and ecology is largely due to a history commercial exploitation, where whalers knew them as "Døglingur" (Faroese), "Anarnaq" (Greenlandic), "Andarnefja" (Icelandic), "Nebbhval" (Norwegian) and "Nordlig døgling" (Danish), and hunted them for their rich oil (North Atlantic Marine Mammal Commission, 2018). Their social behaviour and tendency to approach vessels made them easy targets, and allowed whalers to kill them in large numbers over the last century (Whitehead & Hooker, 2012). As mature male NBW can be over a metre longer than adult females, their sexual dimorphism focuses physical exaggeration on body size, accompanied by pronounced square shaped melons (e.g., Figure 3.1). The large maxillary crests of male skulls, rather overshadows their scant barely erupted teeth (Ellis & Mead, 2017; Gol'din, 2014; MacLeod, 2006). These structural differences in skull morphology even led early taxonomists to diagnose males and females as different species (Ellis & Mead, 2017); with observations of headbutting behaviour proposed as a functional explanation for such displays of weaponry (Gowans & Rendell, 1999).

Similar to other beaked whales, NBW are specialized foragers, diving to extreme depths (800-1400m; Hooker & Baird, 1999) for upwards of an hour in pursuit of their deep-water prey species, primarily squid of the genus *Gonatus* (Hooker et al., 2001). Perhaps due to their energetic demands (New et al., 2013), predictable resource stability (Gowans et al., 2007) and social learning associated with finding localized prey (Gariépy et al., 2014; Whitehead, 2010), NBW have high site fidelity to the Gully, a submarine canyon off Nova Scotia (Gowans et al., 2000). Of the 160 individuals with reliable markings identified from high quality photos taken between 1988-2019, 66% (n = 106) have been seen in more than one year. Almost one-half (45%) of these whales have been seen in 10 or more years, including five individuals (three females and two males), with sighting histories spanning 29 - 30 years (Feyrer et al., 2021). Presumed to be adults when they were first sighted in 1989-1990, the residency of these individuals in the Gully is approaching the documented life span of NBW (37 years; Benjaminsen & Christensen, 1979) and could represent the first generation born after the whaling era. Relatively social compared to other species of beaked whales (Baird, 2019), NBW in the Gully can regularly be found in mixed-sex groups averaging 3-4 (SD = 2.3, max size = 24), with larger group sizes associated with the presence of mature males (mean = 5.5, SD = 3.0) and calves (mean = 4.8, SD = 2.9) (Feyrer, unpublished data). The overall sex ratio of NBW on the Scotian Shelf determined using genetic methods is close to 1:1 (Females = 47, Males = 41) (Feyrer et al., 2021). However, there is some evidence that over the period 1988-2019 that the proportion of mature males has increased, based on the photo-identification of melons (Appendix A, Figure A1a, see also Yeung, 2019) and sightings (Appendix A, Figure A1b). The maturing demographics of NBW on the Scotian Shelf may be an indication of the population’s recovery from the impacts of whaling.

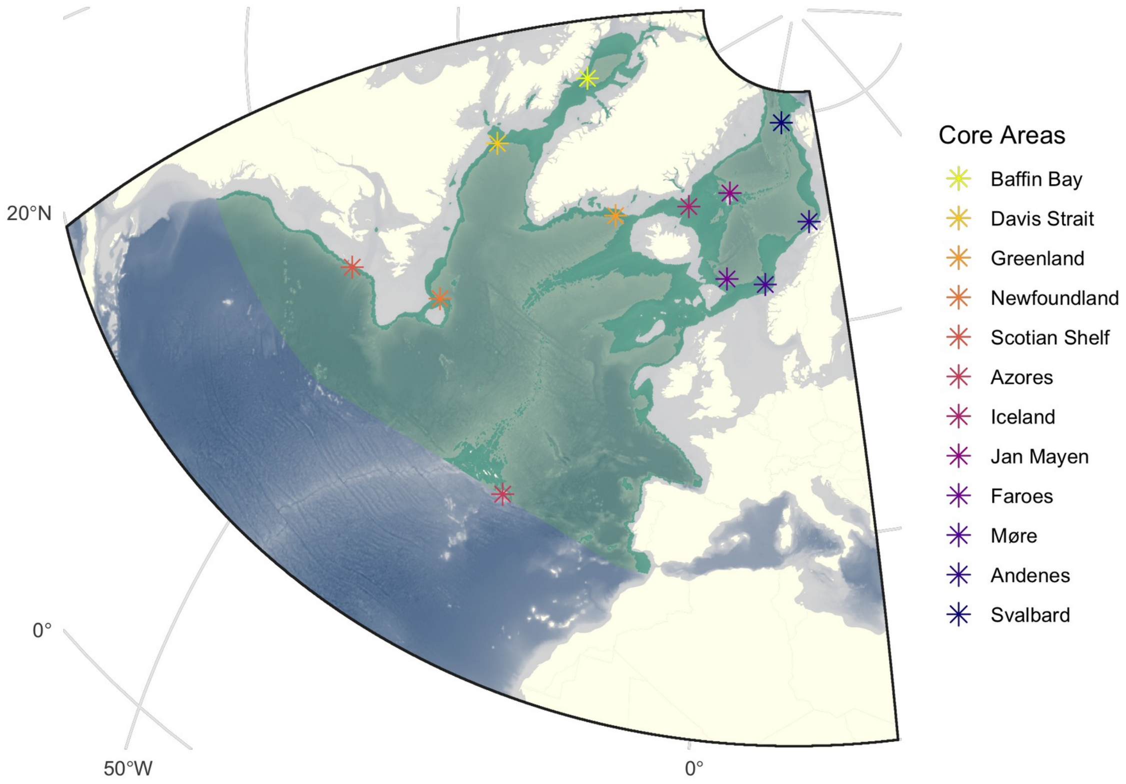


Figure 1.1 General distribution of northern bottlenose whales in the North Atlantic (light green), shown by waters greater than 500 m deep and north of 35°N. Preferred habitat (500−2000 m deep) is shown in dark green. The core areas of NBW observations, including centres of historical whaling operations are indicated as points: (dark orange) Scotian Shelf, Newfoundland; (yellow) Baffin Bay, Davis Strait, Northern Labrador and; (orange) East Greenland; (pink) Iceland, Jan Mayen, Faroe Islands; (purple) Møre, Svalbard, Andenes; and (red) the Azores. Map uses a Lambert azimuthal equal-area projection.

Selecting a suitable model organism to address broad research questions will always be limited by biological (e.g., evolutionary adaptations, life history, ecology), practical (e.g., accessibility and cost) and historical (e.g., previous research) considerations. However, when it comes to the population dynamics of beaked whales, *Drosophila melanogaster* or zebrafish (*Danio rerio*) are hardly analogous models. Beaked whales pose unique questions for species conservation, and NBW have often been used as the de facto model organism to answer them due to the history of whaling and field research, and relative accessibility to known concentrations. The morphological and behavioural peculiarities of NBW somewhat limit the extent of analogies with other ziphiids; however, there are few better options when it comes to representing the range and diversity of traits characteristic of the weirdly wonderful family of beaked whales. In conducting research on NBW I was presented with an opportunity to consider how different methods, patterns and processes could be broadly relevant to other species of beaked whales and cetaceans, while generating meaningful answers for an endangered species, which is still recovering from a legacy of human impacts.

1.3 Thesis Objectives and Key Findings

My thesis proposed to address the following objectives: (1) explore definitions, patterns and processes of population structure found across studies of cetaceans; (2) investigate the population structure for NBW in Canada using molecular methods and contemporary samples collected from across their range in Canada; (3) determine ontogenetic trends in diet using stable isotopes from a collection of NBW teeth to establish weaning age and reproductive potential; (4) assess the prevalence, reliability and frequency of naturally identifiable marks and evidence of anthropogenic interactions with fisheries and vessels using photographic analyses of NBW; (5) estimate the population size and trends for the Scotian Shelf population using photographic identification and mark-recapture methods over the last 30 years; (6) consider long term trends in NBW abundance in the context of cumulative human impacts as part of an evaluation of conservation efforts.

In my field work, I conducted acoustic transects for northern bottlenose whales along the Canadian shelf edge (~1000m isobath), from the Hague line (US-Canada maritime border) to the Straits of Belle Isle (northern tip of Newfoundland). I found detections of NBW scattered along the shelf edge with higher concentrations near the critical habitat areas of the Gully, Shortland and Haldimand Canyons, as well as along the Sackville Spur off Newfoundland (Figure 1.2). Through genetic analysis of samples collected from the Scotian Shelf, Newfoundland and the Davis Strait, and an examination of photo-identification catalogues compiled for each area, I found evidence that Scotian Shelf NBW should continue be managed as a distinct population. However, due to small sample size and no resights of individuals with other areas, we were unable to determine whether NBW in Newfoundland should be considered a unique management unit or as part of a larger more connected population that exists at higher latitudes (Feyrer et al., 2019; Stewart, 2018).

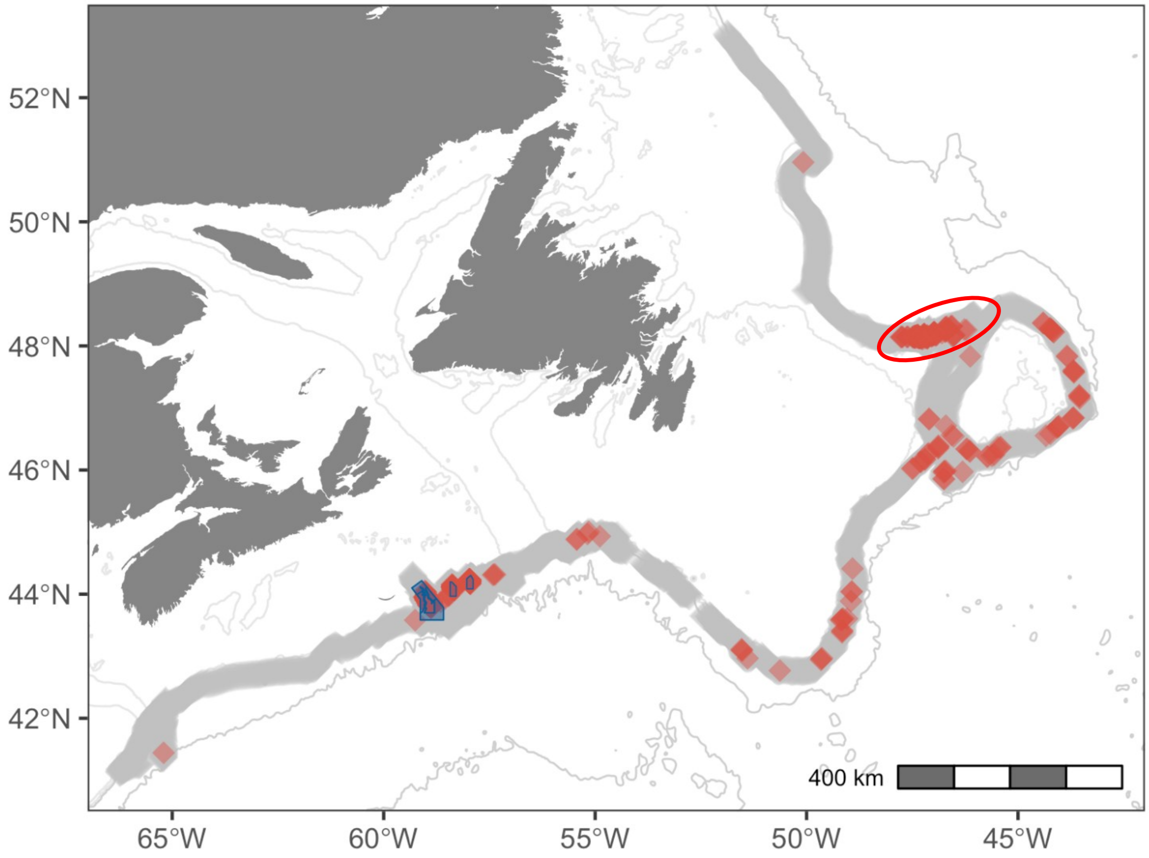


Figure 1.2. Map of the distribution of validated NBW detections (red diamonds) from acoustic surveys (grey shaded line) along the slope edge of Canada and Newfoundland in 2015-2019. Biopsies and photographs were only collected from the Gully MPA and critical habitat areas in Shortland and Haldimand canyons (outlined in blue), the Sackville Spur concentration (circled in red) and the Davis Strait, which was not part of the acoustic survey (see Figure 3.2 for location).

In the process of collecting tissues for genetic analyses we were given over 70 teeth specimens from NBW killed off Labrador and Iceland between 1967-1971, which using stable isotope analysis, offered a rare chance to explore ontogenetic differences in their diet. In contrast to previous estimates, I established a new line of evidence that NBW have prolonged maternal investment, as isotopic enrichment of Nitrogen (N_{15}) from milk continues for three to four years. As NBW are unlikely to care for more than one calf at a time, this effectively reduces female reproductive capacity by 50%.

My reanalysis of the photo-identification catalogue of Scotian Shelf NBW determined that the proportion of individuals with reliable marks slightly increased between 1988-2019, which has implications for mark-recapture estimates. More concerningly, I discovered that scarring consistent with incidents of entanglement and/or vessel strikes indicated rates of anthropogenic interactions in NBW were more than five times the potential biological removal (PBR). A mark-recapture assessment of population trends and an analysis of sightings rates for NBW indicates that between 1988 and roughly 2007, the Scotian Shelf population was declining, but since that time, coincident with the implementation of a marine protected area (MPA) in the Gully, numbers have begun to increase. An analysis of cumulative human impacts across the region suggests that the MPA has been effective in reducing the intensity of threats faced by NBW and should be considered a success in promoting NBW recovery.

1.4 Research Context

My PhD research builds on a long-term field study in the Gully, the largest submarine canyon in the western North Atlantic (~2300 km^2), which Hooker et al. (1999) identified as having the highest density of NBW along the Scotian Shelf. Initial abundance estimates by Whitehead, Faucher, et al. (1997) suggested that the population was quite small, roughly 200 animals, likely less, as Canadian whalers based out of Blandford, Nova Scotia killed at least 87 NBW off the Scotian Shelf between 1962-67 (Whitehead & Hooker, 2012). The end of whaling and the field study also followed in the wake of offshore oil and gas exploration on the Scotian Shelf, dating back to the late 1950's it would eventually result in a number of significant discoveries and petroleum production off nearby Sable Island in the late 1990's (Department of Fisheries and Oceans, 2005). These developments increased conservation concerns for the potential impacts of noise and chemical pollution on NBW and other whale species in the Gully

(Hooker et al., 1999). In light of potential threats related to shipping and oil and gas development and their small population size, NBW in the Gully were assessed as "Vulnerable" by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 1996 (Whitehead, Faucher, et al., 1997). The Gully was also the target of two voluntary conservation measures including an industry led shipping exclusion zone and a Department of Fisheries and Oceans "Whale Sanctuary" in this same period, in an effort to reduce ship traffic, pollution and noise impacts on NBW (Hooker et al., 1999). Although some voluntary measures have proven to be effective in mitigating threats to whales (e.g., ship-strike; Vanderlaan & Taggart, 2009), with few other protections in place, in 2002, the same year that Canada's *Species at Risk Act* (*SARA*) was enacted, COSEWIC reassessed NBW and designated the Scotian Shelf population as Endangered (Fisheries and Oceans Canada, 2016). Although COSEWIC had previously (1996, 2002) assessed the status of the Scotian Shelf population as a separate Designatable Unit (DU), distinct from the NBW inhabiting the region of Labrador-Davis Strait, evidence for the distinction of two populations was limited, relying primarily on geographic separation and an assessment of differences in length distributions (Whitehead, Faucher, et al., 1997; Whitehead, Gowans, et al., 1997). However, in 2001, Dalebout et al. published the first genetic study supporting the management of the Scotian Shelf NBW as a separate population based on significant F_{ST} of four haplotypes across 45 specimens, which included DNA from whaled animals in Northern Labrador.

As COSEWIC is an independent committee of experts, their status assessment reports to government are issued as recommendations and considered as part of the Minister's decision to add species to Schedule I of *SARA* (i.e., the List of Wildlife Species at Risk) at a later date. It was not until the Gully was designated as an *Ocean's Act* marine protected area (MPA) in 2004, that there were any comprehensive or enforceable legal protections for NBW or their habitat outside the Marine Mammals Regulations of the *Fisheries Act*. COSEWIC's status assessments of NBW were influential in the establishment of the Gully MPA, as it was their recognition of the conservation concerns for NBW as well as the diversity and abundance of other cetacean species which helped the unique habitat of the Gully meet the criteria for an *Ocean's Act* MPA (Fisheries and Oceans Canada, 2017; Hooker et al., 1999). The MPA was designed to protect all the habitats within its boundaries, and regulations reflected an assessment of the vulnerability of ecosystems and species, with three nested management zones restricting different activities. The status and distribution of NBW provided the rationale for the highest level of protection in the deep waters (> 500m) of Zone one, excluding all extractive activities including hook and line fisheries, which are otherwise permitted in Zones 2 and 3 (Fisheries and Oceans Canada, 2017). Restrictions to oil and gas, dumping and bottom trawling throughout the MPA were largely already in place when the *Ocean's Act* was updated in 2019 to exclude these activities from all current and future MPAs (Government of Canada, 2019). While the Gully does not exclude shipping or activities related to "national security, sovereignty and public safety", it is identified as an area to be avoided in annual Notices to Mariners (NOTMAR) and in practise the military has said it does not use the Gully for naval exercises, suggesting that these potential threats have been mitigated for NBW in the Gully (Fisheries and Oceans Canada, 2017).

In 2006, NBW were recognized as Endangered under Canada's *SARA*, and became entitled to additional legal protections and a mandate for Fisheries and Oceans to draft a recovery strategy identifying their critical habitat. Identified critical habitat, defined as habitat that is key to the survival and recovery of a species at risk, is protected under *SARA* from "destruction." For aquatic species critical habitat is typically defined based on areas known to be important for foraging, reproduction, socializing and resting, with protections involving permits and restrictions to mitigate the impacts of projects requiring environmental assessments (e.g., oil and gas exploration or development). However, similar to critical habitat designations under the US *Endangered Species Act,* designation does not require specific management actions for critical habitat, and as regular activities, such as fishing or shipping, which are not considered "destructive" are still permitted, so these areas are not equivalent to a habitat reserve or marine protected area (Mullen et al., 2013).

In the same year NBW were recognized under *SARA*, a follow up study by Dalebout et al. (2006) provided stronger support for the distinction of the Scotian Shelf population as a DU, using a larger sample size (n = 161) and additional nuclear markers, though still largely relying on whaling samples from the Davis Strait and notably only finding one additional haplotype. In the meantime Wimmer & Whitehead (2004) had broadened the extent of the study, recording the regular movements of NBW between the Gully and nearby Shortland and Haldimand canyons, sites that would later become recognized as critical habitat in the 2010 Recovery Strategy (Fisheries and Oceans Canada, 2016). The significance of these three submarine canyons for NBW was further demonstrated by acoustic recordings of consistent year-round NBW presence in all three canyons (Moors, 2012; Stanistreet et al., 2017) corresponding with a global pattern of submarine canyons as important habitat areas for cetaceans (Moors-Murphy, 2014). Modelling by Gomez et al. (2017) identified depth and sea-surface temperature as significant predictors of northern bottlenose whale habitat, suggesting that much of the continental slope off the Scotian Shelf, Newfoundland, and Labrador could be potentially suitable habitat for the species. These studies recommended the need to protect habitat connectivity and supported a new analysis of recent acoustic recordings and updated movement rates between critical habitat areas in the canyons (DFO, 2020). I analyzed photo-identification data collected between 2001 and 2017 to update models of residency and movement patterns within and between the canyons in the four data collection years since Wimmer & Whitehead (2004). Results did not find support for individual preference for specific canyons, but were otherwise largely consistent with previous studies demonstrating a high degree of connectivity, as all whales appear to move regularly between canyons over periods of days to months (published in DFO, 2020). As a result of these findings, the deep water areas between the Gully, Shortland and Haldimand canyons are now recognized as important habitat, a step towards being identified as additional critical habitat under *SARA* by Fisheries and Oceans Canada (DFO, 2020).

1.5 Research Scope and Needs

The stated goal of the NBW Recovery Strategy, first finalized in 2010, is "*To achieve a stable or increasing population and to maintain, at a minimum, current distribution"* (Fisheries and Oceans Canada, 2016). The strategy further identifies three research oriented objectives targeting the major knowledge gaps in our understanding of northern bottlenose whales, which include: (1) the ecology, including critical habitat requirements, carrying capacity, breeding, trophic interactions, links with other populations (e.g., Davis Strait), and sources of mortality; (2) the population size, trend and distribution; and (3) anthropogenic threats, including fishing gear interactions, petroleum development, noise, and contaminants. The scope of my thesis largely reflected the objectives of the NBW recovery strategy as I have focused on supporting the management of NBW and their recovery through research that addresses major knowledge gaps and questions related to population structure, size and trends, reproductive potential, and anthropogenic threats. While drawing on data and insights provided through the long-term study in the Gully, my work extended beyond the Scotian Shelf population and considered the case of NBW in Canada. My research is relevant for the recovery of the species and more broadly the global conservation of beaked whales.

When I began my research our understanding of the distribution, movements and population structure of NBW was limited to in-depth studies of the scales of movement within the Scotian Shelf population (Hooker, 1999; Wimmer & Whitehead, 2004) and low resolution genetic analyses. The field of molecular genetics evolves rapidly and the studies conducted by Dalebout et al (2001, 2006), which were based in a large part on historical samples from the Davis Strait and distinguished between populations on the basis of the absence of one haplotype, required revisiting given current thinking on evolutionarily significant management units. While Newfoundland has had a number of NBW strandings over the last two decades (Appendix A Table A1), and recent acoustic recordings confirm their regular presence in the area (Delarue et al., 2018), the concentration of NBW we found while surveying off Newfoundland had not been previously described. The location of the Sackville Spur, midway between the population centres for the two designated units currently identified by COSEWIC, presents new questions for the designation and management of NBW populations in Canada.

Our understanding of the reproductive life history of beaked whales has been largely limited to data collected from stranded specimens (Mead, 1984), whaled animals (Benjaminsen & Christensen, 1979) or more recently, inferences made based on length of behavioural associations with calves (Baird, 2019). For NBW, estimates were based on fetal growth curves indicating gestation lasted 12 months and the stomach contents of a single one year old calf killed by whalers, which resulted in a two year inter calf interval (Benjaminsen & Christensen, 1979). This estimate has been perpetuated through the literature and left unresolved questions on beaked whale reproductive strategies and energetics (e.g., Huang et al., 2011; New et al., 2013). New data and methods were required to move our understanding of beaked whale life history forward.

Long-term field studies are an ongoing source of important empirical datasets that can be used to address some of the gaps, however they only exist for a few species of beaked whales in addition to NBW, namely Blainville's (*Mesoplodon densirostris*), Cuvier's (*Z. cavirostris)*, and Baird's (*Berardius bairdii*) beaked whales (Hooker et al., 2019). Photo-identification catalogues, such as those for the Scotian Shelf NBW, which began in 1988, have begun to answer questions on social structure and population trends (e.g., Gowans et al., 2001; O'Brien & Whitehead, 2013). Although catalogues are rich resources for longitudinal analyses, the evolution from small film datasets to exponentially growing digital libraries can challenge old information management strategies. The transition to working with larger digital catalogues offers an important opportunity to revaluate methods, assumptions, error rates and ask new questions, such as whether there is evidence for anthropogenic interactions in scaring patterns.

The recovery of small populations can be delayed due to intrinsic limiting factors (e.g., slow reproduction, genetic limitations, alee effects) or extrinsic human threats (e.g. activities resulting in direct or indirect mortality), and population level responses to the end of whaling or any large demographic event needs to be considered at the scale relevant to life history. While field data collection for NBW on the Scotian Shelf started close to one generation post whaling (i.e., roughly 17.6 years; Taylor et al., 2007), data collection on the population since has just approached the scale of two generations. Evaluating the considerable conservation efforts made to reduce threats for NBW on the Scotian Shelf over the last 15 years is necessary to understand how different levels and types of protected areas, from critical habitat, marine refuges, and no-take MPAs can mitigate threats associated with human activities. Understanding the efficacy of protected areas and other management strategies could inform new measures, which may determine the survival of future generations of NBW and beaked whales around the world.

My thesis addressed the research needs outlined above using established and innovative methods to analyse and interpret historical, novel and modelled datasets. The following section provides an overview of each of my chapters in terms of their methods, key findings and importance, identifying how they are related to each other and support my overall thesis objectives.

1.6 Summary of Thesis Chapters

Species management relies on a definition of the management unit, which is often below the species level. In Chapter two, I considered the question of sub-specific population structure in cetaceans through a systematic literature review of 356 studies conducted between 1982-2018 and a meta-analyses of 120 studies on odontocetes. I outlined the range of definitions used by authors for distinguishing a ‘population’ and consider how these relate to evolutionary and ecological patterns and processes in the marine environment. I provided a framework for understanding the linkages between legal and biological population concepts and identified how these ideas fit within management and conservation objectives. I highlighted how the definitions of evolutionarily significant populations and units used to manage cetaceans depend on jurisdiction, scale and study objectives. In a metanalysis of odontocetes, I summarised trends across studies and evaluate the opportunities, challenges, limitations and recommendations for research that can improve our appreciation of cetacean population structure. Overall, I found most species of cetaceans demonstrated some degree of population structure, however differences in objectives, scale and methods preclude more significant phylogenetic comparisons. This review highlighted how theoretical allopatric, parapatric or sympatric patterns and processes of sub-specific population structure are found across cetaceans, however there are many species where such structures have yet to be considered. In my reflection on future directions I advocate for clarity, in identifying the underlying evolutionary or ecological definitions of population structure to support a comparative understanding of trends for management, across taxa and within the field of population biology.

While different levels of intra-specific structure can be determined through a number of methods, determination of evolutionarily significant population structure is now dominated by the field of molecular genetics. Genetic tools have widely been used across conservation biology not only to understand the distinctions between and within species, but also to understand the evolutionary potential and genetic risks due to low genetic diversity and impacts of past demography. In Chapter three, I used molecular genetic methods to assess the population structure, genetic diversity and historical demography for NBW across the western North Atlantic. I expanded the temporal and spatial distribution of sampling, using new contemporary samples from across the range of NBW in Canada, including samples from the Sackville Spur, an area in international waters off Newfoundland, and increased the resolution of genetic markers from previous analysis by Dalebout et al. (2001, 2006). Despite low diversity across the mitogenome and 37 novel microsatellites, I found that individuals in the Scotian Shelf population appear to share a unique genetic inheritance distinct from NBW in other areas. The results presented in this chapter, and published by Feyrer et al. (2019), have improved our understanding the genetic patterns and evolutionary processes influencing NBW, confirming their management as a separate designated unit. While the history of exploitation is not shared across the Ziphiid family, beaked whales are highly adapted predators, which can increase their site fidelity, isolation from other populations and the genetic vulnerability of their small populations.

The timing and rate of reproduction are critical life history traits for understanding limitations on population growth and recovery for species at risk. In Chapter four, I used stable isotopes of nitrogen (δN^{15}) and carbon (δC^{13}) recorded in the annual layers of NBW teeth to investigate the diet signatures related to the ontogeny of weaning age and nursing duration. I discovered evidence of prolonged maternal investment that changed our understanding of the reproductive interval for NBW from two to four years, effectively halving lifetime reproductive potential. Significantly for NBW, the combination of long generation times (Taylor et al., 2007) and prolonged maternal care will impact population growth and expectations of recovery (from historical whaling or other chronic or acute demographic events) need to be adjusted. Due to a paucity of empirical data on beaked whale reproductive traits, the results of this chapter published by Feyrer et al. (2020), has additional implications for estimates of reproductive output and energetics for other species of beaked whales (e.g., Huang et al., 2011; New et al., 2013) as many of these models were based in part on the previous understanding for NBW inter-calf intervals.

Although whalers stopped killing NBW in Canada in 1971, their recovery in the face of genetic risks and low reproductive capacity, may have been further limited by threats including fisheries entanglement, shipping, military sonar, climate change and oil and gas exploration and development activities that have been ongoing across their range. Monitoring the impact of these threats in offshore areas is challenging, however photo-identification data has played an important role in estimating the size and trends in cetacean populations over time. In Chapter five, I reassessed the prevalence and stability of natural and potential anthropogenic scars over thirty years using a photographic catalogue of individual NBW from the Scotian shelf. In contrast to previous analyses (Gowans & Whitehead, 2001; O'Brien & Whitehead, 2013) that occurred over shorter time periods, only dorsal fin notches and back indents were found to be reliable for photoidentification purposes over the 30 years of the study. More concerning was the analysis of anthropogenic scars which indicated that the threat of entanglement and propeller-vessel strikes has been ongoing at a steady rate with these scars being observed on 6.6% of the population. With an annual gain rate five times the accepted potential biological removal (PBR) for the Scotian Shelf population, the results presented in this chapter and published by Feyrer et al. (2021) suggest the risks posed by entanglement are higher than generally assumed, requiring action and further study.

NBW are a long-lived species and population monitoring has been ongoing for almost two generations. In Chapter six, I considered how trends observed in the abundance of NBW estimated using long-term sightings and photo-identification data reflect the spatial and temporal dynamics of human impacts and conservation areas across their pelagic habitat. I found that while NBW abundance was steadily declining between 1988-2004, this trend subsequently reversed and began increasing, coincident with the implementation of the Gully MPA in 2004. From my analysis of change in the cumulative human impacts (CHI) across the study area, it appears the comprehensive regulation of threats within Zone one of the Gully were effective in decreasing CHI in a small but important area of core habitat for NBW. This study has important implications not only for whales and other species with high site fidelity, but also for the implementation, management and evaluation of pelagic MPAs.

In conclusion, my final chapter, Chapter seven, demonstrates how the research I conducted contributed to my thesis aims and objectives and can be used to inform management and future research in the field of beaked whale conservation biology.

Chapter 2 Cetacean Population Structure for Conservation and Management: A Review

2.1 Publication Status

As of 27 July 2021, this chapter is in review. Submited as Feyrer, L.J. Cetacean population structure for conservation and management: A review. Marine Mammal Science.

2.2 Abstract

Following the end of commercial whaling, there has been a major effort to understand patterns of population structure in whales and dolphins. Despite a lack of obvious boundaries and their large dispersal potential, cetaceans have frequently demonstrated population structure. Ecological and evolutionarily significant population units are broadly used terms to manage and assess the status of cetaceans. This review outlines the diversity of definitions, patterns, processes, and lines of evidence studies use to describe population structure and considers how they inform the conservation and management of cetaceans. A systematic literature review of 356 studies on population structure between 1982-2018 found research was uneven across species. The majority of studies focussed on coastal species in the family Delphinidae (45%), largely *Tursiops* sp. (21%), primarily using genetic methods (64%). A detailed meta-analysis of odontocetes (N = 120 studies, 37 species) described allopatric, parapatric and sympatric patterns and processes. Population structure (k > 1 population) was found in 90% of odontocete studies, average k = 2.8 units (range 2-12). Structure varied across genera and habitat, as well as elements of study design, inhibiting broader biological generalizations. Despite hundreds of studies, large knowledge gaps still disproportionately affect cetaceans that are currently threatened or data deficient. Consideration for ecological mechanisms and the evolutionary significance of patterns would increase the impact of future research for conservation and management.

2.3 Introduction

The aim of this review is to summarize our current understanding of cetacean population structure and explore how this area of research has contributed to species conservation and management. Marine mammals exhibit relatively low overall species diversity. Extant species include about 90 cetaceans, 33 pinnipeds, and four sirenids (Committee on Taxonomy, 2021) many fewer than most other animal clades including birds (18,000; Barrowclough et al., 2016), terrestrial mammals (6,300; Burgin et al., 2018), reptiles (11,000; Uetz & Stylianou, 2018) and marine fishes (17,000; Eschmeyer et al., 2010). Although wide-ranging data-poor species (i.e., many species of cetaceans) are likely underrepresented by strict taxonomic species concepts that require substantial evidence of differentiation (Taylor, Perrin, et al., 2017), six new species of cetaceans have been discovered in the last two decades (Committee on Taxonomy, 2021; WoRMS, 2021). However, the conservation of evolutionarily important biodiversity depends on more than identifying new species or even sub-species. Protecting the adaptive diversity vital to the future resilience of cetaceans largely depends on our ability to identify and manage their populations (Sgrò et al., 2011).

Investigating population structure is inherently linked to the adopted species concept, leaving population structure a similarly contentious subject for debate (Crandall et al., 2000; Taylor, Perrin, et al., 2017; Waples & Gaggiotti, 2006). Terminology and criteria can vary widely between jurisdictions and authors, who provide definitions of significance that range from strict to “fuzzy”, focus on evolutionary or demographic characteristics, apply benchmarks ranging from genetic variation or divergence to ecological connectivity, over scales of evolutionary time or generations (Table 2.1) (Fraser & Bernatchez, 2001). The inconsistencies can challenge interpretations and comparisons, limiting the value of studies to species conservation and management (Dizon et al., 1992; Fraser & Bernatchez, 2001; Waples & Gaggiotti, 2006). While the consensus struggles to come to terms with what is significant, populations continue to be defined and described.

Given the importance of intra-species variation for conservation, the scope of this review focuses on population structure below the sub-species level. It begins by summarising ecological and evolutionary significance and classifying the range of population unit terminology. It follows with an overview of the commonly described patterns and processes of cetacean population structure, giving consideration to differences in study design and trends found from a meta-analysis of studies. I conclude by discussing the opportunities and challenges for studying cetacean population structure and identify recommendations for how the field can better support the scientific management and conservation of cetaceans.

Table 2.1 Definitions of population concepts commonly cited in studies of cetacean population structure and criteria suggested by authors to distinguish them

Concept	Definition	Criteria/ Evidence	Reference
Evolutionarily Significant Unit (ESU)	A set of historically isolated populations that demonstrate reproductive isolation or phenetic similarity. Must be reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci.	Reciprocally monophyletic for mtDNA alleles	(Moritz, 1994, 2002)
	A cluster of organisms possessing at least one unique character or a combination of characters that are "diagnosable" (distinguishable) to the exclusion of other groups. Differences can be any heritable trait: genotypic, phenotypic (morphological) behavioral, or ecological.	Any heritable trait that is diagnosable using cladistic population aggregation analysis	(Vogler & Desalle, 1994)

Concept	Definition	Criteria/ Evidence	Reference
	Populations characterized by a discontinuous genetic divergence pattern, where locally adapted and closely related genome assemblages are separated geographically and by significant genetic distances. A hierarchical categorization of phylogeography based on distinctions of (a) distributions, (b) population level responses, (c) phenotypic characteristics and (d) gene frequencies. "Category I" is ESU level and distinct across (a,b,c,d).	Genetic divergence, genetic distance, and geographic discontinuities considered in a hierarchical classification	(Dizon et al., 1992)
	A population (or group of populations) that is substantially reproductively isolated and represents an important component in the evolutionary legacy of the species. Evaluated based on inferences about historical levels of gene flow that have occurred over evolutionary time scales. Isolation does not have to be absolute, but it must be strong enough to permit evolutionarily important differences to accrue in different population units. The second criterion would be met if the population contributes substantially to the ecological/genetic diversity of the species as a whole.	Isolation based on movements of tagged animals, natural recolonization rates, measurements of genetic differences, or efficacy of natural barriers. Significance based on genetics, unique habitat features or phenotypic adaptations.	(Waples, 1991, 1995)
Management Unit (MU)	Populations where the degree of connectivity is "sufficiently low" to require separate monitoring and management. A level of genetic divergence at which populations become demographically independent.	Genetic divergence, demographic independence	(Palsbøll et al., 2007)
	Areas with restricted interchange of individuals between adjacent geographic areas. The relevant amount of interchange largely depends on the management objectives and risk.	Critical levels of gene flow informed by management	Taylor & Dizon, 1999.
	A group in which local population dynamics are determined primarily by birth and death rather than immigration and emigration. It must be possible to distinguish between continuous and subdivided populations and to identify the subdivisions that exist.	Movement data between identified populations	(Paetkau, 1999)
	A demographically distinct group where local population dynamics are determined primarily by birth and death rather than immigration and emigration and should be managed	Genetic divergence of allele frequencies at an unspecified	(Moritz, 1994, 1994, 1995)

Concept	Definition	Criteria/ Evidence	Reference
	separately to ensure the viability of the larger ESU, regardless of phylogenetic distinctiveness.	number or type of loci	
Demographically Independent Population (DIP)	A group at the lowest level of hierarchical biological organization, whose dynamics are more a consequence of births and deaths within the group (internal dynamics) than of immigration or emigration (external dynamics). An appropriate level of population structure for management.	Demographic evidence	(Taylor, Perrin, et al., 2017)
Stock	A broad term frequently used to describe a variety of subdivided or local populations for analytical convenience; applied in the simplest and least restrictive sense. Little qualification is made or assumed about its genetic, evolutionary, or ecological implications. Can encompass or reference similar terms with more specific definitions (e.g. Phenotypic stocks, genotypic stocks).	Can be genetic, phenotypic or environmental	(Coyle, 1998; Dizon et al., 1992)
Conservation Unit	Based on population distinctiveness for variation in phenotypes, where significance is tested based on a null hypothesis of genetic and ecological exchangeability. Considers functional diversity at both recent and historical timescales to determine management level. Evaluations can range based on criteria from ESU to DIP.	Crosshair analysis of contemporary and historical evolutionary and ecological distinctiveness	(Crandall et al., 2000)
Ecological Population	A species group that co-occurs in space and time so there are opportunities to interact with each other. Cohesive forces are largely demographic and can include competition, social and behavioural interactions, etc. Demographic cohesiveness depends on the rate of immigration from other subpopulations (m).	Threshold for demographic independence $m < 0.1$	(Waples & Gaggiotti, 2006)
Evolutionary Population	A group of individuals living in close enough proximity to permit mating with any other member. Group cohesion is primarily genetic, and emphasis is on reproductive interactions between individuals. Depending on question, population distinctions can be based on levels of departure from Panmixia (any variation or specific thresholds in terms of effective migrants N_{em}).	Threshold numbers of Nem e.g. Nem < 1	(Waples & Gaggiotti, 2006)

2.3.1 Why Care About Cetacean Population Structure?

Due to over-exploitation by past whaling and the impacts of ongoing anthropogenic activities in the marine environment, many species of cetaceans are the focus of conservation concern (IUCN, 2021; Lotze & Worm, 2009). One species is thought have become extinct in the last decade (e.g., the bajji, *Lipotes vexillifer*), and, according to the IUCN's *Red List of Threatened Species (2021), 53%* of the remaining 130 listed species, sub-species or sub populations of cetaceans are designated as Vulnerable (VU), Near Threatened (NT), Endangered (EN) or at Critical Risk (CR) of extinction, and an additional 8% are considered data deficient (DD). With the majority of cetaceans considered at some level of risk of extinction, the conservation needs are pressing and require effective management strategies to ensure recovery.

Cetaceans have been studied at the stock or population level for at least as long as they have been subject to formal management through the International Whaling Commission (IWC), which was formed in 1946. However, the distinction and identity of different whale stocks, or any other organizational level below the species (subspecies, race, deme, evolutionarily significant unit, management unit, etc.) has been an ongoing and controversial issue for the IWC and for the field of conservation biology in general (Taylor, Archer, et al., 2017). Likely due to the preoccupation of science for "discovering" cryptic species and sub-species over the last century, the number of studies on sub-specific diversity in cetaceans has increased over the last 30 years (Figure 2.1).

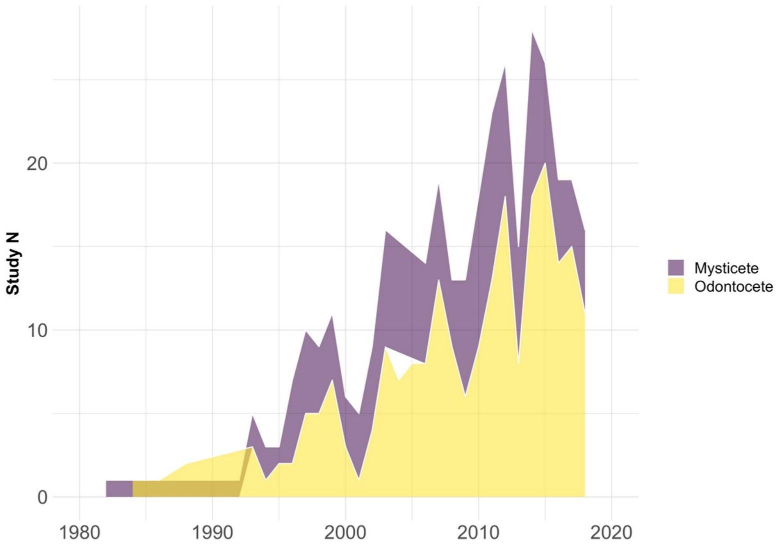


Figure 2.3 Number of studies on cetacean population structure over the last three decades by sub-order

The vast majority (90%) of studies on odontocetes in this review found evidence of population structure, suggesting many cryptic populations likely also contain important genetic, behavioural and cultural diversity, which may not be replaced if lost. In defining and describing populations, we also identify the unit for research or status assessments, and the focus of the majority of management efforts. From determining biologically informed management boundaries and assessing anthropogenic threats, to designing protected areas and evaluating recovery measures, prioritizing populations scales up to serve species level conservation.

2.3.2 What Makes a Population Significant? Definitional Challenges

The importance of intraspecific biodiversity and the evolutionary potential for the conservation of species was broadly recognized by governments following the adoption of the Convention on Biological Biodiversity (CBD, Rio de Janeiro, Brazil, 1992). The resulting need to distinguish levels of structure within the formally recognized species has led to various interpretations of patterns of diversity in light of their ecological or evolutionary significance. One prevalent idea the evolutionarily significant unit (ESU) has been debated and defined using various biological thresholds and criteria (Table 2.1; Fraser & Bernatchez, 2001; Taylor, Perrin, et al., 2017; Whitehead, 2010). For the purpose of this review an ESU is defined as a demographically independent population (DIP) or group of DIPs that demonstrate substantial reproductive isolation from other units and embody an important aspect of the species' evolutionary potential (Sensu Taylor, Archer, et al., 2017). Below the level of ESU, DIPs are populations whose demographics are largely determined by internal processes (births and deaths), rather than emigration or immigration dynamics, but are not substantial enough to suggest reproductive isolation (Taylor, Archer, et al., 2017).

The ESU has become synonymous with a level of biodiversity that should be prioritized for conservation; however, broad understanding of what constitutes an ESU has been challenged by the lack of consensus on an operational definition. Issues that are not biological or even scientific, such as maintaining consistency in legal and management definitions specific to national and international jurisdictions (e.g. between the United States, Canada and Australia and the IUCN), have resulted in a confusing array of nearly synonymous terms for sub-population units (see Table 2.2). Largely as a result of the science-policy interface there are now a diversity of similar ESU type thresholds used to denote cetacean sub-populations, including "stocks" (Booke, 1999), "demographically independent populations" (Taylor, Perrin, et al., 2017), "Management Units" (Waples & Gaggiotti, 2006), "Evolutionarily significant Units" (Moritz, 1994) and conservation units (Crandall et al., 2000).

To help explain the relationship between ESU's and other unit terminology this review provides a typology of "units" categorizing them in two ways: (1) in terms of scale, as having either broad conservation goals or specific management objectives; and (2) with consideration for ecological or evolutionary relevance (Figure 2.2, Tables 2.2, Table 2.3). While conservation and management are often used interchangeably, in this review they are distinguished as having different orientations, applications and scales. In (1) unit terms are distinguished as having either a *conservation* orientation, focusing on largely aspirational broader goals and longer-term priorities across the range of a species or population, or a *management* orientation, which is operational, specific, target driven, applied over smaller areas within shorter time frames. Given that evolution is affected by a number of ecologically significant processes, population unit terms can be further distinguished by the scope of what they consider "evolutionarily significant." In (2) definitional perspectives are distinguished as either: *inclusive*, where a variety of ecological processes and different timescales may be relevant to evolution, and should be considered (Crandall et al., 2000; Palsbøll et al., 2007); or *exclusive*, where only the process of long term biological or reproductive isolation is relevant to evolution (Moritz, 2002).

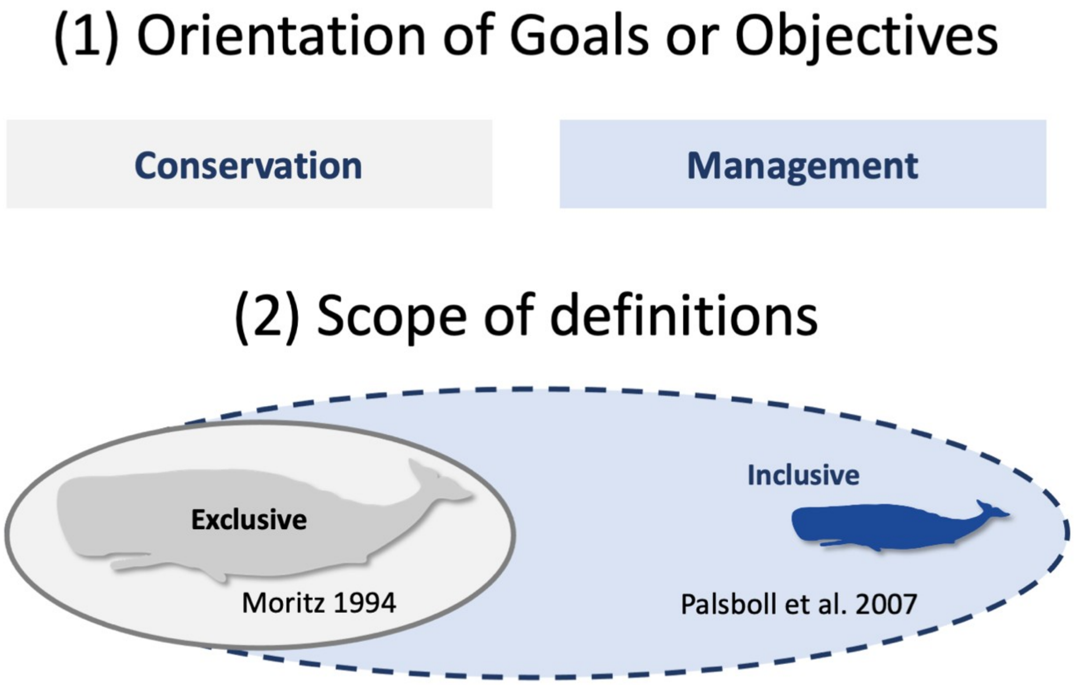


Figure 2.4 A typology for distinguishing population unit concepts and terminology. Units can be classified based on (1) the orientation and scale of the stated goals and objectives, being either Conservation focused (e.g., broader aspirational longer-term priorities for the range of a species) or Management focused (e.g., smaller scale operational targets applied over shorter time frames) and (2) the definitional scope of "evolutionarily significant", which can be exclusive (e.g., only long term biological or reproductive isolation is relevant to evolution; Moritz 1994) or inclusive (e.g., as a variety of ecological processes and different timescales may be relevant to evolution and should be considered; Palsbøll et al., 2007).

Table 2.2 The conservation vs. management objectives, evolutionary vs. ecological significance and inclusive vs. exclusive definitions based on unit term concepts used by authors. More than one term or concept could be used in each study

Term	Concept	Study N	Objective	Significance	Definition
(1)	Evolutionarily Significant Unit (ESU); Evolutionary Unit; Evolutionarily Independent Unit; Taxonomic Unit	17	Conservation	Evolutionary	Exclusive
(2)	Conservation Unit	6	Conservation	Evolutionary	Inclusive
(3)	Biological Population; Isolated Sub-Populations; Reproductively Isolated Unit; Genetic Stock; Genetically Distinct Stock	4	Management	Evolutionary	Exclusive
(4)	Demographically Independent Population (DIP); Demographically Independent Management Units; Demographically Independent Unit; Demographically Distinct Population; Independent Demographic Units	13	Management	Ecological	Exclusive
(5)	Stock; Management Stock	141	Management	Ecological	Inclusive
(6)	Management Unit (MU)	50	Management	Ecological	Inclusive
(7)	Ecological Unit; Ecologically Important Stock; Ecological Management Unit; Ecological Stock	5	Management	Ecological	Inclusive

Table 2.3 A selection of legal tools with significance to cetaceans from jurisdictions that recognize population-level management, including terms, summary definitions.

Legal Tool	Term(s)	Definition	Objective	Significance	Concept
Marine Mammal Protection Act (1972) USA	Stock, MU, DIP	A stock is a group of marine mammals of the same species or smaller taxa in a common spatial arrangement that interbreed when mature. A stock is recognized as being a management unit that identifies a demographically independent biological population.	Management	Ecological	Inclusive
Species At Risk Act (2002) Canada	Designatable Unit, ESU	Designatable Units should be discrete and evolutionarily significant units of the taxonomic species, where "significant" means that the unit is important to the evolutionary legacy of the species as a whole and if lost would likely not be replaced through natural dispersion.	Conservation	Evolutionary	Inclusive
Environment Protection and Biodiversity Conservation Act (1999) Australia	Population	A population is geographically isolated, distinct and able to be defined in a way that differentiates it from all other populations.	Management	Evolutionary	Exclusive
International Union for the Conservation of Nature (IUCN)	Subpopulation	Subpopulations are defined as geographically or otherwise distinct groups in the population between which there is little demographic or genetic exchange (typically less than one successful migrant individual or gamete per year or less).	Conservation	Evolutionary	Exclusive
International Whaling Commission (IWC)	Stock, Taxonomic Units	'Species' [stocks] should be taken to refer to taxonomic units below the species level where appropriate.	Management	Evolutionary	Inclusive

2.4 Overview of Cetacean Population Structure

2.4.1 Meta-analyses

Studies on cetacean population structure were identified using a systematized literature review of two abstract and citation databases (Biological Abstracts and Web of Science Core Collection) and "snowball" sampling of references within those studies (Appendix B, Supplementary methods). All peer-reviewed articles published between 1982 and August 15, 2018 were screened for relevance and 356 were summarized in terms of focal species, geographic area, methods, and keywords from the abstract. Additional details on experimental design, patterns, processes, unit terminology, and goals were extracted through a full text review of 120 studies on odontocetes using standardized coding and definitions (see Appendix B for details). The goal of the meta-analyses was to provide an overview of trends in the field and consider the influence of species-specific characteristics and experimental design on the significance of population structure. While more recent publications have been included as examples, results of analyses are only considered representative for the period 1982-2018. Other sources of data used in the meta-analyses, such as the taxonomy of cetaceans and IUCN Redlist status are based on the 2021 publication year.

2.4.2 Processes and Patterns

In studies of cetaceans, there are at least 10 patterns of population structure that have been described, varying by degree of population connectivity, genetic distinctiveness, which align with concepts of allopatry, parapatry and sympatry (Figure 2.3). With the exception of sympatric patterns, where distinctions occur due to behavioural, cultural or niche separation, most concepts in cetacean population structure are common across a wide range of taxa (Funk et al., 2006). Using examples from the literature on cetaceans, in the following section I summarize the concepts of (1) panmixia, (2) sub-species, (3) the island isolation model, (4) isolation by distance, (5) isolation by environmental distance, (6) clines, (7) stepping stone, (8) metapopulations, (9) ecotypes and (10) cultural clans, with consideration for species and processes associated with these patterns (Table 2.4).

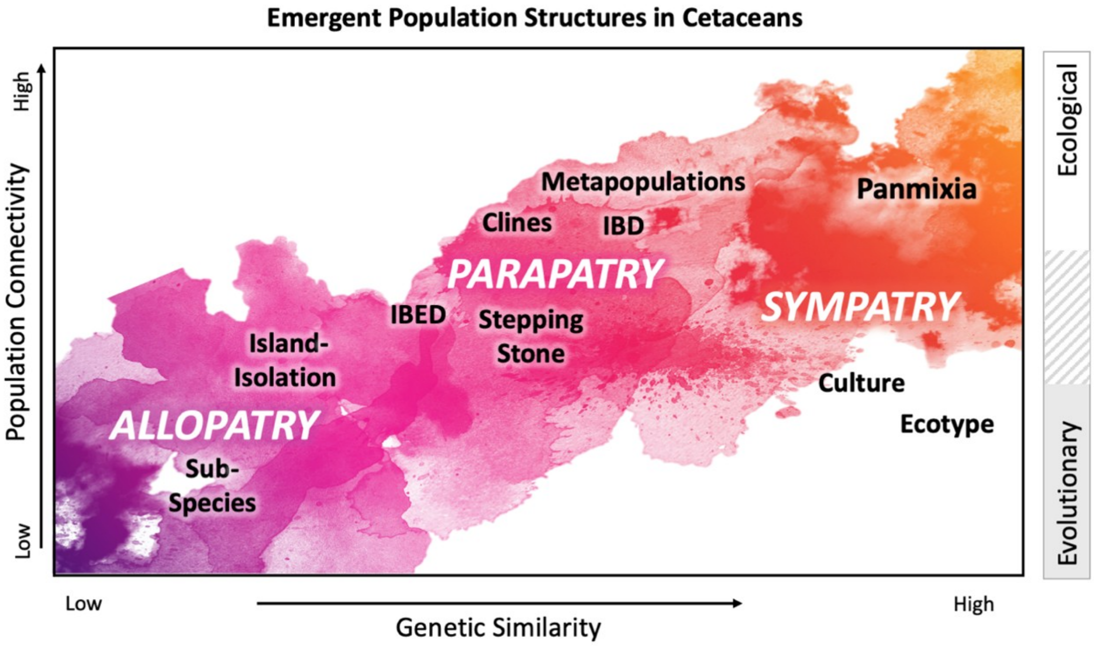


Figure 2.5 Concept map identifying the continuum of patterns and processes driving emergent population structure in cetaceans. X axis represents degrees of genetic similarity between populations. Left y axis represents degrees of connectivity or potential dispersal between populations and right y axis represents distinctions between evolutionary and ecological drivers. Theoretical patterns of population structure align with the underlying allopatric, parapatric, and sympatric domains and overarching evolutionary – ecological drivers. IBD = Isolation by Distance, IBED = Isolation by Ecological Distance.

2.4.2.1 Allopatry

Allopatric patterns are distinguished by low connectivity and increased genetic distinctiveness, often occurring as the result of a significant geographic barrier to dispersal. In the marine environment barriers to dispersal and discontinuous habitat may occur over evolutionary time frames (e.g., continental drift, vicariant landscape events, glacial processes, shallow continental shelves and deep-water canyons) resulting in refugia and population isolation (Hewitt, 1996). Contemporary ecological processes that can isolate habitat include stable oceanic fronts, steep clines in temperature, pH, salinity and productivity that can aggregate prey and predators, creating areas of high site fidelity.

Similar to “Category I Populations” described by Dizon et al. (1992), allopatric patterns include sub-species and populations explained by the island-isolation model, which were identified in 23% of studies in the full text review of odontocetes. Although the detection of subspecies is not a focus of this review, they are defined here as an upper limit for further discussion of other population structure typologies. To paraphrase the definition provided by Taylor, Archer, et al. (2017), cetacean subspecies are considered a taxonomic unit below species that appear to be a separately evolving lineage due to forces restricting gene flow and result in populations that are “diagnosably distinct.” As of 2018, there were between 51 and 59 subspecies recognized by the Society for Marine Mammalogy’s Committee on Taxonomy (2018). This included the Black Sea harbour porpoise sub species *Phocoena phocoena relicta,* which using genetic and morphological data, Viaud-Martinez et al. (2007) found to be distinct and reproductively isolated from other harbour porpoises (*Phocoena phocoena)* in the North Atlantic, Mediterranean and the Black Sea.

The model of island biography is a widely applied thought experiment in population ecology that considers dynamics based on differing degrees of connectivity (MacArthur & Wilson, 1967). The island-isolation pattern illustrates the case where due to extinctions of intermediary populations or geographic barriers, a population has low connectivity and little chance of genetic rescue. Because isolation processes may be recent or ephemeral, or due to lack of data, these populations do not (yet) qualify under taxonomic criteria as a subspecies. In Viricel et al. (2016), Spinner dolphins (*Stenella longirostris*) off the coast of Zanzibar, east Africa, were found to have significant genetic population structure between three sites in the Mascarene archipelago. Viricel et al. (2016) attributed these distinctions to geographic isolation and habitat discontinuities in their shallow water habitat requirements.

2.4.2.2 Parapatry

Differences can still occur between adjacent cetacean populations without complete isolation through parapatric patterns or processes including isolation by distance (IBD), isolation by environmental distance (IBED), clines, stepping stone and metapopulations. Some form of parapatry was described for 36% of studies on odontocetes, with patterns resulting from the distribution and density of suitable habitat, regional adaptations or dispersal limitations. Populations are typically characterized by moderate-low genetic differentiation, with some evidence of recent divergence, such as local adaptation, morphology or demographic separation. Most of these distinctions would be characterized by Dizon et al. (1992) as "Category III Populations", and either Archetype II (Stepping Stone) or III (Diffusion-Isolation by Distance) in the IWC (2004) schema, however there is considerable overlap in the literature surrounding each of these typologies, so each will be defined and briefly described with an example for consistent understanding within this review.

IBD was first described by Wright (1943) who proposed that the genetic distance between populations is a function of their geographic separation, constraining dispersal limitations and population size (Sexton et al., 2013). The hypothesis of IBD is simplistic, but widely influential on other theories considering distance-based patterns of spatial population structure. IBD was found to explain the relatedness of juvenile, but not adult harbour porpoise in Fontaine et al.'s (2017) genetic study of population structure of porpoises that stranded around the UK.

IBED, builds on IBD and describes the correlation between environmental heterogeneity, local adaptations, non-random mating and population structure (Mendez et al., 2010). Under IBED genetic relatedness between similar environments is higher than predicted by IBD alone (Sexton et al., 2013) and synonymous with analyses sometimes referred to as "IBD with environmental correlates" and "seascape genetics" (Amaral et al., 2012). In a study of Franciscana dolphins in Argentina (*Pontoporia blainvillei*), Mendez et al. (2010) found that genetically isolated populations were significantly correlated with environmental conditions distinguished by remote sensing data on chlorophyll, turbidity and sea surface temperature (SST).

Clines, another variant of IBD, describe a pattern of consistent change in gene frequency or heritable phenotype, but typically refer to a single trait or loci. Clines can be gradual, occurring across the entire range of a population (e.g., equivalent to IBD), or steep and restricted to smaller areas of transition between sub-populations, also referred to as hybrid zones (Hewitt, 1988). Clines are continuous patterns, reflecting underlying environmental conditions and usually defying further division. For common dolphins off Mauritania, Pinela et al. (2011) correlated the rostral lengths and stable isotopes of δ^{15}N with a cline, suggesting that niche segregation was driving the structure of their population.

The stepping stone model of population dynamics is a classic, first described by Kimura & Weiss (1964) to understand dispersal between adjacent populations. In contrast to continuous or clinal patterns, the stepping stone model describes a series of divergent populations across a landscape. Conceptual variations of the model may specify the dimensions of movement within the population, including the order or progression of "steps" and directionality (Kimura & Weiss, 1964; Palsbøll et al., 2007). In a study by Hamner et al. (2012), the distribution of Hector's dolphins (*Cephalorhynchus hectori)* around the South Island of New Zealand) displayed a step-wise directional gene flow between populations along the east and west coasts that suggested sex-biased dispersal of males.

Metapopulations tie the ideas of IBD, IBED, the island model and stepping stone patterns into a theory that has grown in complexity and application since Levin's (1969) original "population of populations". More of a heuristic process than a defined pattern, metapopulations typically describe population dynamics that are influenced by the spatial distribution of habitat across multiple scales (Grimm et al., 2003; Hanski, 1998; Kritzer & Sale, 2010). Studies that consider multiple variables including population connectivity, isolation, patch /population size and the distribution of habitat to inform conclusions on population structure, invoke concepts relevant to metapopulation processes. In a global study of melon-headed whales *Peponocephala electra* (MHWs), Martien et al. (2017) determined that despite residency around oceanic islands and strong social structure, evidence of low genetic differentiation indicated that MHW had occasional long-distance dispersal and/or gene flow with larger pelagic populations, similar to a metapopulation structure.

2.4.2.3 Sympatry

Patterns of sympatry, where distinct populations occur within the same area (e.g., ecotypes or cultural clans), can occur due to differences in site fidelity, culture and niche dimension (Whitehead, 2010) and were found in 33% of studies on odontocetes. Sympatry can occur across a range of levels of site fidelity or philopatry, from seasonal populations in maternally associated foraging or breeding areas (e.g. Humpback Whales; Baker et al., 2013; Carroll et al., 2015), to lifelong matrilineal associations, where natal or kin-based social structures limit group dispersal (e.g. killer whales, sperm whales, Hoelzel et al., 2007; Gero et al., 2008). Dizon et al. (1992) considered such cases as a “Category II” populations, where despite sympatry, critical differences in some combination of behavior, morphology or genetics indicate a degree of reproductive isolation.

The ecotype is a broad classification describing a population adapted to local climatic, resource or other conditions, and would be less fit in other parts of the species range or niche (Morrison et al., 2012). In contrast to clinal patterns, ecotypes are distinguished by multiple trait adaptations across a composite of environmental conditions. Given their proposed significance as forms of incipient or potential speciation (Lowry, 2012), ecotypes have been widely examined across a range of other taxa including other mammal (e.g., woodland caribou (*Rangifer tarandus)*, Courtois et al., 2003) and bird species (e.g., great tits (*Parus major),* Caizergues et al., 2018). In cetaceans, ecotypes are broadly associated with inshore-offshore or coastal-pelagic ecosystems (Louis et al., 2014), resident-transient behaviour (Martien et al., 2017), and trophic specialization such as fish-eating and mammal eating forms of killer whales

(Filatova et al., 2015; Hoelzel et al., 2007; Morin et al., 2015). Ecological specialization in cetaceans may occur due to cultural reinforcement (e.g. Killer Whales, Whitehead & Ford, 2018), however not all ecotypes demonstrate evidence of significant differences in social structures or cultural patterns.

Culture in animals, defined as a socially learned group behaviour (e.g., Whitehead & Rendell, 2015), is a process that can determine population structure. Culture can result in selection for phenotypic patterns, typically mirroring social networks, and can, in some cases lead to reproductive isolation. Culturally distinct behaviours, such as vocal clans, can occur in sympatry with the result that population structure is not spatially expressed (Whitehead, 2010). Culturally-driven population structure also appears in the absence of obvious niche specialization, such as in the case of sperm whales (Rendell et al., 2012), humpback whales (Murray et al., 2011), and southern right whales (Carroll et al., 2015). Social conformity and other ecological processes may also act to reinforce cultural patterns of divergence (Whitehead, 2010; Whitehead & Ford, 2018).

2.4.2.4 Panmixia

At the other end of the spectrum from allopatry, investigating population dynamics often relies on panmixia as a null hypothesis. Widely assumed for mobile marine species, including cetaceans, it is also known as the idealized population, where random mating and a lack of reproductive isolation or barriers results in strong connectivity and within group similarity (Palsbøll et al., 2007). This pattern was described in 8% of studies of odontocetes. Corresponding to Dizon et al.'s (1992) “Category V Population” and the IWC’s (2004) “Archetype I”, panmictic populations are distinguished by a lack of internal structure ($K = 1$), with minimal differences in morphology, genetics, or demographic parameters between neighbouring populations. That panmixia occurs at some scale below the boundary between subpopulations is a hypothesis used to test putative boundaries in multiscale analyses. Thompson et al.'s (2016) study found genetic panmixia across samples of Gray’s beaked whales (*Mesoplodon grayi*) stranded around New Zealand, and suggested the pattern was likely due to a combination of abundant habitat, expansion or a selective sweep and a lack of behavioural barriers or evidence for modular social structure.

Table 2.4 Patterns and processes of population structure found in Cetacea as described or inferred from a review of Odontocete studies (N= 120). * indicates where the number of populations (k) = 1 was detected.

Structure descriptions	Study n	Mean k	Max k
Allopatry	28	2.8	5
Island Isolated	14	2.6	4
Geographic Barrier	14	2.8	5
Parapatry	44	3.0	9
Breeding/Foraging	4	3.3	6
Cline	2	2.5	3
IBD	14	2.7	7
Metapopulation*	8	3.1	6
Stepping Stone	6	4.0	9
IBED	10	3.0	6
Sympatry	39	3.1	12
Cultural*	3	3.0	5
Eco-type	23	3.4	12
Philopatry*	13	2.9	6
Panmixia*	9	1.0	1
Total	120	2.8	12

2.4.3 Investigating Cetacean Population Structure

Studying population structure in cetaceans involves the measurement and analysis of different lines of evidence for signals of group connectivity or division. Recognizing divisions that occurred over evolutionary time typically involves genetic or morphometric analyses, while investigations of ecological or cultural processes may assess differences in trophic level or acoustic repertoires. The following section provides an overview of the application of different methods giving consideration for the strengths and limitations of methods for investigations of cetacean population structure (Table 2.5).

Table 2.5 The strengths and limitations of different methods that can be used as primary (used independently or on the majority of samples) and/or secondary (used in addition to another method) lines of evidence in investigations of population structure.

Method	**Line of Evidence**	**Strengths**	**Limitations**
Acoustics	Primary or Secondary	Evolutionary patterns (cultural) in vocalizations between sympatric, parapatric or allopatric populations and ecological patterns in spatial or temporal habitat use, distribution or movement.	Requires large sample sizes. Uncertainty regarding temporal/ seasonal variability and dialect evolution. Expensive but becoming increasingly cost-effective with bottom-mounted and roving recorders, and automated analysis.
Contaminants	Secondary	Ecological patterns of population structure for species in sympatry or parapatry.	Uncertainty regarding the temporal residency of contaminant markers, trophic pathways and utility for pelagic or migratory species. High cost/ sample.
Demography	Secondary	Evolutionary or Ecological patterns suggesting reproductive independence, low migration or other population distinctions.	Requires long term detailed datasets to identify natural variation and plasticity. Easier for smaller populations or geographic areas.
Distribution and Range	Secondary	Evolutionary or Ecological patterns that inform hypotheses and experimental strata for primary methods. Identify extent of range overlap between populations.	Uneven survey effort in some areas may fail to detect range extent, or structure within migratory patterns.
Fatty acids	Secondary	Ecological patterns of population structure for species in sympatry or parapatry.	Dependent on accurate prey baselines. Physiology may affect assumptions. High cost/sample.
Genetics	Primary or Secondary	Evolutionary patterns of divergence and isolation. Historical inferences on the timing of lineage separation of allopatric populations.	May not be able to distinguish more recent divergence, populations in sympatry or parapatry with low levels of gene flow. Software models and assumptions can be complex to parameterize and interpret.
Habitat	Secondary	Ecological patterns related to environmental processes. Inform experimental strata for primary methods.	Relies on availability and quality of secondary datasets for environmental correlates. Scale of habitat drivers may not match primary data or methods.
Morphology	Primary or Secondary	Evolutionary patterns in phenotypic variation between populations. Can identify differences between specimens that exist in allopatry or parapatry.	Requires large sample sizes to distinguish population vs. plasticity or individual level variation. Spatial providence of specimens may be inadequate. Can indicate ecological function or cline rather than isolation.
Parasites	Secondary	Ecological patterns for allopatric or ecotype populations. Identify	Dependent on understanding of parasitology and pathways. Stranded

Method	Line of Evidence	Strengths	Limitations
		vagrants from different climates or ecosystems.	specimens may not be unbiased representatives of regional variation.
Pigmentation/ Fin shape	Secondary	Evolutionary or Ecological patterns. Contemporary variation between allopatric, parapatric or sympatric populations. Non-invasive to document for live specimens.	Requires large sample sizes to distinguish population vs. individual level variation. Can indicate ecological cline rather than significant isolation.
Photo-ID	Primary or Secondary	Ecological patterns. Detect demographic differences, site fidelity and trends for sympatric, parapatric or allopatric populations. Identify spatial or temporal patterns in habitat use, distribution or movement. Useful for social structure analyses which may support Evolutionary patterns.	Depends on well-marked individuals. Requires long term field studies and detailed datasets. Better for smaller populations or regional scale analyses.
Social Structure	Secondary	Evolutionary patterns. Detect degrees of association and social complexity that can indicate emergent cultural patterns. Can inform hypotheses, experimental strata and interpretation (e.g. genetic analyses).	Depends on well-marked individuals and long term photo identification datasets. Better for smaller populations or regional scale analyses.
Stable Isotopes	Primary or Secondary	Ecological patterns (ecotypes) of population structure for species in sympatry or parapatry. Low cost/ sample for $\delta^{13}C/\delta^{15}N$.	Dependent on accurate understanding of regional trophic baselines to compare allopatric distinctions. Need estimates for all potential prey SI values for trophic level. Sample sources and storage can prevent comparative analyses.
Stomach Contents	Secondary	Ecological patterns. Inform hypotheses on ecological patterns of population structure for species in sympatry or parapatry.	High temporal sensitivity. Requires large sample sizes to distinguish population vs. individual level variation. Prey species may be underrepresented. Stranding sourced specimens may be biased indicators.
Sightings/ Stranding rates	Secondary	Ecological patterns. Inform hypotheses on spatial or temporal patterns in habitat use, distribution or movement. Can use opportunistic and historical data.	Sightings for deep diving/ offshore species may not indicate true extent of habitat use. Stranding specimens may be vagrants or biased indicators.
Tags	Secondary	Ecological patterns. Inform hypotheses and experimental strata for primary methods. Indicate contemporary scales of habitat use, distribution or movement. Identify extent of range overlap between populations.	Expensive and invasive to deploy on a per sample basis. May not be appropriate for Endangered populations. Individual variation may bias population level interpretation.

Method	Line of Evidence	Strengths	Limitations
Whaling Catch Records & Discovery Tags	Secondary	Ecological patterns. Inform hypotheses and experimental strata for primary methods. Indicate historical scales of habitat use, distribution or movement. Identify potential overlap between populations.	Bias in data reliability over time and between regions. Emphasis on commercially important species.

2.4.3.1 Genetic Methods

Across taxa, genetics, which first appeared in studies of cetacean population structure in 1993 is now the method of choice for exploring the range of patterns and scales of population structure, as 63% of all studies primarily used genetic methods (Figure 2.4, Table 2.6). Advances in genetic techniques over the last two decades have increased the power and decreased costs of analyses; studies can now use thousands of markers to detect very low levels of genetic differentiation (Cammen et al., 2016). However, the pace of genetic advances used in cetacean research has yet to catch up, as of 2018 in genetic studies of odontocetes, 73% used multiple markers, typically combining microsatellites (average of 7 loci) with 400-500 bp of the mitochondrial control region, with fewer than 10% of studies using higher resolution markers such as SNPs (n = 5) or mitogenomes (n = 1) (Table 2.7).

Table 2.6 The 12 primary study methods used in investigations of cetacean population structure from a review of 356 studies from 1982-2018 with the subset of those included in the odontocete review indicated in parentheses (n). For primary methods that were also used in combination with secondary methods, the range of secondary methods is indicated. Average number of populations (k) is reported based on primary method from the review of odontocetes where Study n > 1.

Primary Method	Study n	Secondary Methods	Average k
Acoustic	18 *(7)*	Photo ID	3.6
Contaminants	5 (2)	-	2.0
Fatty acids	2 (1)	-	na
Genetic	227 *(80)*	Acoustic, Contaminants, Demography, Distribution, Fatty Acids, Habitat, Morphology, Parasite/ Pathogen, Phenotype, Photo ID, Social Structure, Stable Isotopes, Tags	2.9
Morphometrics	14 *(7)*	Demography, Genetic, Habitat, Phenotype, Stable Isotopes	2.1
Parasites	1	-	na
Photo-identification	57 (10)	Distribution, Range Social Structure	2.3
Sightings	1	-	na
Stable isotope	19 *(9)*	Morphology, Contaminants, Habitat, Stable Isotopes	2.3
Stomach contents	3 (2)	-	3.0
Stranding rates	1	-	na
Tagging	2 (1)	Genetic	na
Whaling data, Discovery Tags	6 (1)	-	na
Total	356 (120)		2.8

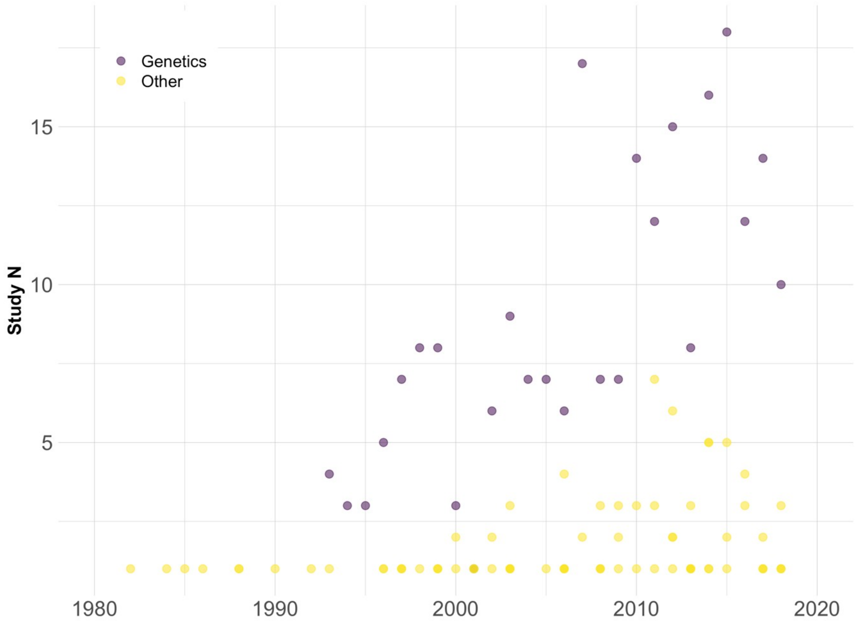


Figure 2.6 The use of genetic versus other methods over time across all studies of cetacean population structure

As genes are directly inherited markers that can quantify the extent of reproductive isolation between populations, genetics may be the most relevant method for answering questions of evolutionary adaptation and diversity. This has led to the influential proposal that genetics should be the exclusive method of diagnosing population structure with the significance determined by reciprocal monophyly of mtDNA haplotypes (Moritz, 1994; Palsbøll et al., 2007). Although a genetic test for reproductive isolation may appear biologically relevant and straightforward, genetic methods do have limitations. Low FST, or slight differences between the mean pairwise distances may be interpreted as significant structure, even when the vast majority of diversity is within populations rather than between them (Granot et al., 2016). Conversely panmixia may be incorrectly diagnosed if genetic methods fail to detect low levels of structure in parapatric (e.g., stepping stone or metapopulations), or sympatric populations (e.g., ecotypes or cultural clans) despite potentially important distinctions (Crandall et al., 2000; Fraser &

Bernatchez, 2001; Taylor & Dizon, 1999; Whitehead, 2010). With higher resolution (especially genomic) data there is the potential to detect and date ongoing divergence, local extinctions (i.e. ghost populations; Estoup & Guillemaud, 2010) or demographic events (Feyrer et al., 2019), however, for long-lived cetaceans, recent impacts on genetic variation, such as those from commercial whaling may not yet be apparent (Phillips et al., 2013). Finally, genetics may not be able to provide clarity on the underlying mechanisms, as similar patterns can occur via very different processes, such as panmixia, which can result from regular or sporadic recruitment across distant areas, or stabilizing selection in similar environments, or recent divergence (Coyle, 1998).

Table 2.7 Markers used in genetic studies and average k population structure. mtDNA control region (CR) were the most popular marker used (n = 65), followed by microsatellites (n =52). All markers were used in combination and on their own.

Marker resolution	Study n	Mean k	Microsat mean (n)	SNPs mean (n)
mtDNA CR < 300bp	4	3.8	11.5	
mtDNA CR < 400bp	13	3.8	14.5	39.0
mtDNA CR < 500bp	28	3.0	11.0	1002.3
mtDNA CR > 500bp	21	2.8	12.3	
cytochrome b	9	2.8		
microsatellites	52	3.1	12.2	
mitogenome	1	12*		91
SNPs	5	5.5		
Total	80	3.1	12.2	627.4

* Not a mean value as study n = 1.

2.4.3.2 Other Lines of Evidence

Although morphology remains a significant tool for classifying the diversity of traits within and between species and was used in 5% of studies of odontocetes, it can be prone to error when superficial similarities or differences exist and leave room for debate as to plasticity versus heritability (e.g., reservations regarding the Burrunan dolphin (*Tursiops australis)*; (Charlton-Robb et al., 2011; Jedensjö et al., 2020; Moura et al., 2020). Understanding ecological, contemporary and sympatric patterns and processes typically requires lines of evidence other than genetics or morphometrics. Landings and discovery tags, a tenet of the whaling era for stock delineation were the primary method in less than 2% of all studies (e.g., Mizroch & Rice, 2013), and have since been replaced by less harmful tracers that can record site fidelity, as well as the range, scale and seasonality of movements. Methods such as satellite tags (0.6% of studies, e.g., Bloch et al., 2003; Prieto et al., 2014), passive acoustic monitoring (5% of studies, e.g., Delarue et al., 2009; Hoffman et al., 2017), stable isotope modeling (5% of studies, e.g., Trueman et al., 2019), sightings (0.2% of studies, e.g., Van Waerebeek et al., 2013), stranding rates (0.2% of studies, e.g., McLellan et al., 2002), parasites (0.2% of studies, e.g., Iwasa-Arai et al., 2018), traditional ecological knowledge (0.2% of studies, e.g., Perrin et al., 1996), behavioural and photo-identification (16% of all studies, e.g., Baird et al., 2009; Mahaffy et al., 2015) have been used to infer, delineate or support investigations of population structure (Reeves et al., 2004). Because differences between sympatric ecotypes or cultural populations may not possess strong genetic footprints (but see Carroll et al., 2015; O'Corry-Crowe et al., 2010; Rendell et al., 2012), identifying patterns and processes often relies on other lines of evidence. Investigating trophic differences or prey preferences of putative ecotypes typically involves an examination of foraging behaviour using either stable isotopes (5% of all studies, e.g., Ruiz-Cooley et al., 2011), fatty acids (0.6% of studies, e.g., Walton et al., 2008), contaminants (1.4% of studies, e.g., Reiner et al., 2011), and/or stomach contents (0.8% of studies, e.g., Denuncio et al., 2017). Although not typically the primary line of evidence, analyses including acoustic communication repertoires (e.g., identity calls; Hersh et al., 2021), social structure (e.g., Fearnbach et al., 2014), association indices (e.g., Carnabuci et al., 2016) and site fidelity (e.g., Alves et al., 2013) using photo-identification and behavioural methods, can help identify culturally transmitted population structure (Whitehead & Rendell, 2015).

Our understanding of the strengths and limitations of various methods (Table 2.7) for detecting the diverse expressions of population structure has grown with 30% of all studies employing multiple techniques and tests, presumably to increase the likelihood of discerning structure (Crandall et al., 2000; Dizon et al., 1992; Moritz, 2002; Paetkau, 1999). Using multiple methods has provided new insights regarding killer whale ecotypes

(Filatova et al., 2015) and cultural patterns in sperm whales (Rendell et al., 2012), but has largely relied on descriptive comparisons, or the use of novel priors in Bayesian techniques for genetic analyses. Currently there are no established methods for integrating multiple lines of evidence into a single modelling framework (but see Rundel et al., 2013). The idea that combining different methodological approaches could increase analytical power to reveal different scales or patterns of structure is appealing, particularly given the challenges in studies of cetaceans. However, using multiple lines of evidence still requires consideration for the pattern(s) of interest, gradients in the distribution of data and differences in the temporal scales of inheritance between genes and other markers, issues which can make results challenging to interpret (Monteiro et al., 2015; Rundel et al., 2013).

2.4.3.3 Questions of Experimental Design and Scale

As emphasized throughout this review, determination of significant population structure is context dependent and most relevant to the specific question under consideration. Given the potential implications of structure for management and conservation, interpretations must be measured against the limitations of available data, methods, assumptions, pattern of interest and life history of the species. Unfortunately, robust sample sizes collected from temporally and spatially balanced strata across the region of interest are not the typical case for cetaceans, leaving many aspects of research design to chance. However, analytical choices, such as whether it is appropriate to predetermine the boundaries of putative populations (e.g. using STRUCTURE, (Hubisz et al., 2009)), and the validity of pooling temporally distinct data (e.g., from seasonal feeding and breeding areas or historical whaling era and cotemporary collections) needs to be carefully considered and tested for bias.

Patterns of population structure can also vary depending on the temporal or spatial scales of analysis and patterns that appear at meso-scales may not be present at macro-scales (and vice versa). For cetaceans, study regions and management areas are often small relative to oceanic gradients or the size of a species' range. That said, some cetacean species have demonstrated extremely fine scale population structure on the order of 10-100 km (e.g., Hector's Dolphins, Guiana Dolphins, and Bottlenose Dolphins; Fazioli et al., 2006; Hollatz et al., 2011; Weir & Sagnol, 2015). Small scale studies have also motivated and contributed to broader scale investigations across the range of the species (e.g., killer whales, Morin et al., 2015; melon headed whales; Martien et al., 2017). Multiscale analyses have been used to understand whether patterns of population structure translate to different scales of analysis (e.g., Pratt et al., 2018; Viricel & Rosel, 2014).

2.4.4 Trends Across Studies of Odontocetes

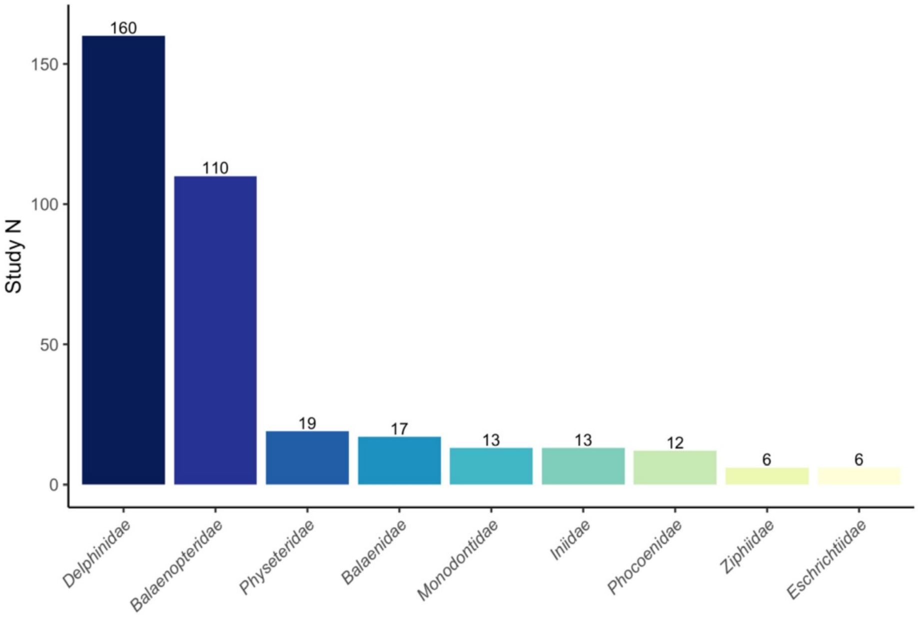
It appears that much of what we have learned about cetacean population structure defies panmictic assumptions derived from a general absence of marine boundaries. In fact, panmixia is the exception rather than the rule, as population structure seen across 90% of odontocete studies included nearly equal proportions of allopatric, parapatric and sympatric patterns and processes, finding two or more populations on average (mean k = 2.8). Population structure was found to vary across odontocete genera, with *Orcinus, Peponocephala, Sousa*, *Stenella* and *Delphinapterus* having more populations than average (Table 2.8), however, differences in the proportion of studies focussing on Delphinidae (62%) relative to other families constrains interpretation of further biological or phylogenetic trends (Figure 2.5). Perhaps intuitively, the number of populations detected (k) increased with multiple habitat types (Table 2.9), but also with larger scale study areas ($p < 0.001$, Figure 2.6), putative populations (i.e., the number of populations described by studies as the null hypothesis, $p < 0.000$, Figure 2.7) and multiscale analyses (mean k = 3.25). While use of particular lines of evidence or consideration for multiple methods did not appear to affect the number of populations detected (Table 2.7), attempting to compare results highlighted how the scale of objectives, inclusive or exclusive definitions of significance and some elements of study design can impact what is detected and described as population structure in cetaceans.

Table 2.8 Summary of average number of populations (k) found by genera, where more than one study was available and k > 1. *Indicate genera where no structure (k= 1) was also reported.

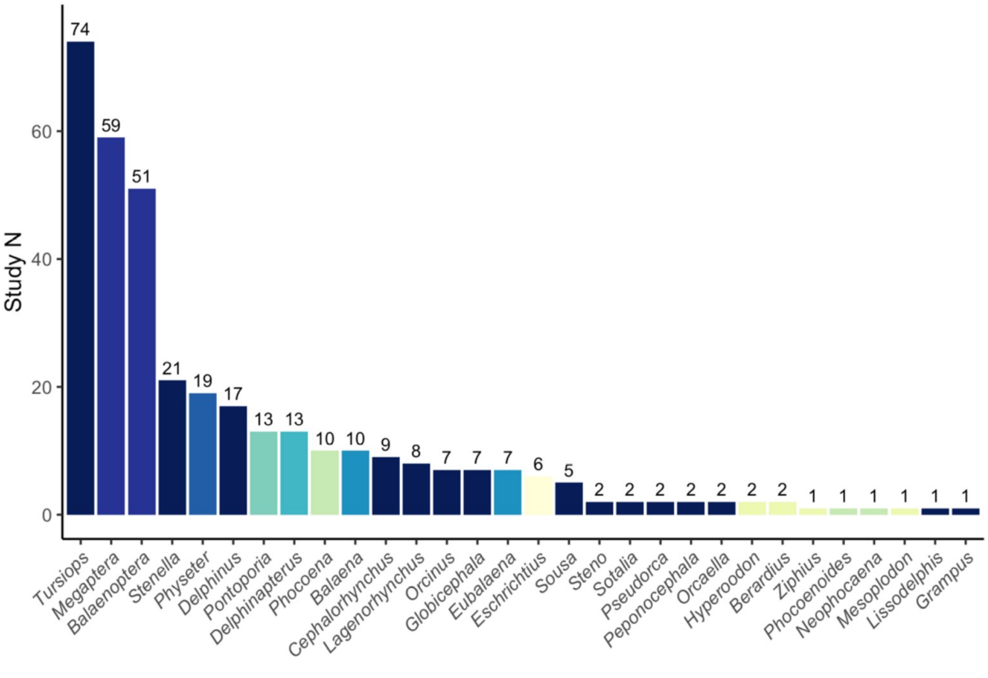
Genus	Study n	Average k
Orcinus	6	5.5
Peponocephala	2	3.5
Sousa	5	3.4
Stenella	8	3.1
Delphinapterus	8	3.0
*Tursiops**	13	2.9
*Pontoporia**	11	2.7
*Cephalorhynchus**	7	2.7
*Globicephala**	7	2.7
*Delphinus**	14	2.6
Orcaella	2	2.5
*Phocoena**	8	2.5
*Physeter**	12	2.4
Lagenorhynchus	5	2.4
Hyperoodon	2	2.0

Table 2.9 Average number of populations (k) by habitat class from review of 120 studies of odontocetes.

Habitat	Study n	Mean k	SD k	Max k
Pelagic & Coastal	21	4.0	2.8	12
Coastal	64	2.8	1.2	7
Pelagic	22	2.4	1.2	5
Shelf	7	2.4	0.5	3
Estuary	3	2.7	1.2	4
River	1	na	na	1
River vs. Coastal	2	2.5	0.7	3



(a)



(b)

Figure 2.7 The representation of studies (N) from the full review of cetacean population structure abstracts by (a) Family and (b) Genus. Study N by Genus in (b) are coloured by cetacean Family.

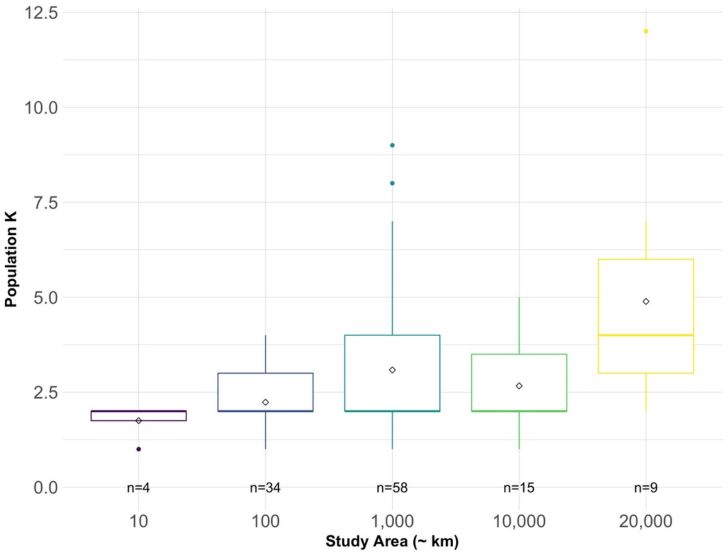


Figure 2.8 Population structure found in odontocete studies by scale of study area. Scale was classified based on the maximum distance between sample sites in km.

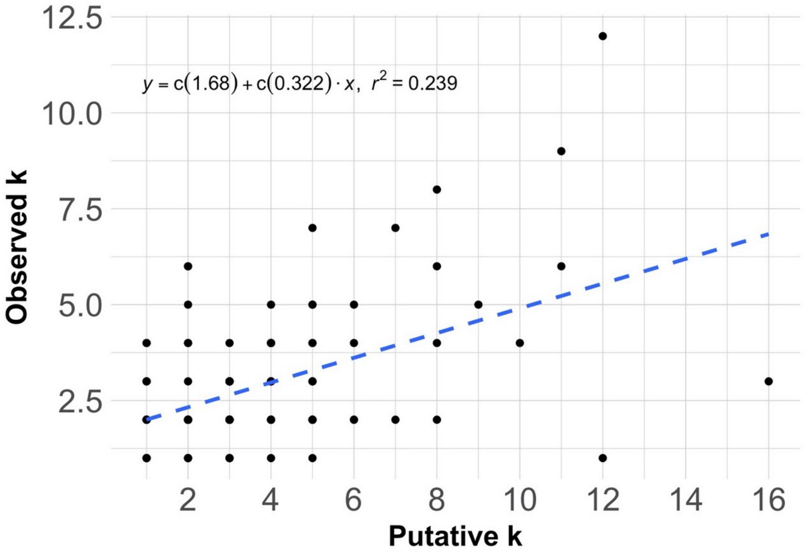


Figure 2.9 The number of observed populations (k) determined by studies in relation to putative number of populations

2.5 Opportunities and Challenges for Conservation

The history of research in this field reflects the evolution of scientific discourse on what constitutes a biologically meaningful population, advances in our understanding of cetaceans, and new scientific methods. Scientific perspectives have also clearly influenced the relevant management paradigms and policies for the conservation of cetacean populations, demonstrating the importance of clearly communicating research implications for focal species. However, this review could say little about the population dynamics of nearly half of the 90 currently recognized species of cetaceans without published studies (as of 2018) or the ~25 species with only one or two studies available (Table 2.10). In the two years since the meta-analyses was conducted the SMM Taxonomy Committee continues to recognize new species and sub-species, while the updated IUCN Red List now includes assessments for 28 sub-populations of cetacean species, of which 70% are Critically Endangered or Endangered. As other meta-analyses have also emphasized (Kaschner et al., 2012; Lotze & Worm, 2009; Parsons, 2016), until recently, a near majority of cetacean species were classed as data deficient (e.g., 45% in IUCN 2018). In the 2021 red list assessment, the change in status of many cetaceans reflects updated guidelines (IUCN Standards and Petitions Committee, 2019) with only 8% of cetaceans now considered data deficient. While this helps identify where we have made advances in our knowledge of species status, our understanding of population substructure has not kept pace. If populations are the practical unit for management this remains a critical gap in advancing the conservation and recovery of species.

Table 2.10 The species included in meta-analyses and their 2021 IUCN Red List Status. Some species have subspecies or sub-populations with different status assessments as indicated. Where EN - Endangered, VU - Vulnerable, NT - Near Threatened, LR - Lower Risk/conservation dependent, LC - Least Concern, DD - Data Deficient. Species without assessments are indicated as na. * *Tursiops australis* is currently not a recognized species or subspecies, see reservations noted by the Committee on Taxonomy (2021).

Genus	Species	IUCN Status	Study n
Balaena	*mysticetus*	EN/ LR/ LC	10
Balaenoptera	*acutorostrata*	LC	18
Balaenoptera	*borealis*	EN	3
Balaenoptera	*edeni*	LC	6
Balaenoptera	*musculus*	EN	13
Balaenoptera	*musculus brevicauda*	na	1
Balaenoptera	*physalus*	VU	10
Berardius	*bairdii*	LC	2
Cephalorhynchus	*commersonii*	LC	3
Cephalorhynchus	*eutropia*	NT	2
Cephalorhynchus	*heavisidii*	NT	1
Cephalorhynchus	*hectori*	EN	3
Delphinapterus	*leucas*	LC	13
Delphinus	*delphis*	LC	17
Eschrichtius	*robustus*	EN/ LC	6
Eubalaena	*australis*	CR/ LC	4
Eubalaena	*glacialis*	EN	3
Globicephala	*macrorhynchus*	LC	4
Globicephala	*melas*	LC	3
Grampus	*griseus*	LC/ DD	1
Hyperoodon	*ampullatus*	NT	2
Lagenorhynchus	*acutus*	LC	1
Lagenorhynchus	*albirostris*	LC	1
Lagenorhynchus	*obliquidens*	LC	2
Lagenorhynchus	*obscurus*	VU/ DD	4
Lissodelphis	*borealis*	LC	1
Megaptera	*novaeangliae*	EN/ LC	59
Mesoplodon	*grayi*	LC	1
Neophocaena	*phocaenoides*	VU	1
Orcaella	*brevirostris*	CR/ EN	2
Orcinus	*orca*	CR/ DD	7
Peponocephala	*electra*	LC	2
Phocoena	*phocoena*	CR/ LC	8
Phocoena	*phocoena relicta*	EN	1
Phocoena	*spinipinnis*	NT	1

Genus	Species	IUCN Status	Study n
Phocoenoides	*dalli*	LC	1
Physeter	*macrocephalus*	EN/ VU	19
Pontoporia	*blainvillei*	VU	13
Pseudorca	*crassidens*	NT	2
Sotalia	*guianensis*	NT	2
Sousa	*chinensis*	CR/ VU	4
Sousa	*sahulensis*	VU	1
Stenella	*coeruleoalba*	VU/ LC	7
Stenella	*frontalis*	LC	7
Stenella	*longirostris*	VU/ LC	7
Steno	*bredanensis*	LC	2
Tursiops	*aduncus*	NT	1
Tursiops	*australis*	na	1
Tursiops	*truncatus*	CR/ VU/ LC	71
Tursiops	*truncatus ponticus*	EN	1
Ziphius	*cavirostris*	VU/ LC	1

Overall our understanding of cetacean populations has been limited by an uneven distribution of research effort across species, ocean basins, habitats and jurisdictions (Kaschner et al., 2012). In this review the majority of investigations focused on only two species: bottlenose dolphins and humpback whales. Globally, field research for marine species suffers from geographical biases in the economic availability and allocation of funding, as well as nationally restricted management interests (Magera et al., 2013). The geographic distribution of research reviewed here was no exception, as the majority of studies occurred within coastal or nearshore environments (Table 2.9), and in the jurisdiction of high income western economies (Appendix B, Figure B1). Issues of proximity, accessibility and risk aversion continue to challenge field studies of cryptic species (e.g., beaked whales; Hooker et al., 2019), in remote offshore areas and in habitats bordering low and middle-income countries. However, public interest and support for the conservation of whales and dolphins suggests that these species and areas also represent opportunities for future research effort and investment (Naylor & Parsons, 2018).

These knowledge gaps indicate that not only is there still room to investigate other cetacean populations and regions, but there is a need to continuously revisit unit terms and criteria based on technological advances, other lines of evidence and the development of new methods to improve the field of research. Overall, population structure was primarily investigated using genetic methods, and in the case of odontocetes, 70% of the studies used genetics exclusively, which may not be relevant to contemporary ecological processes. Given that sympatric patterns may be hard to detect genetically but prevalent in cetaceans, other approaches are likely required to identify cultural or ecological populations (Whitehead, 2010). However, some challenges, such as biases in sampling or experimental design cannot be overcome with technological solutions. Consistent across scales and species ranges, the majority of studies in this review inferred population structure, typically at k~ 2. Such a pattern could reflect known issues with the genetics program STRUCTURE for detecting k = 2 in datasets with uneven sample sizes (Janes et al., 2017; Puechmaille, 2016), or a symptom of positive-outcome bias, where studies are more likely to report or emphasize the magnitude of an effect (Fanelli et al., 2017).

This review revealed some important geographical, species and methodological biases, identifying important areas for improvement and challenges that will require considerable shifts in effort, funding and interest.

2.6 Limitations and Recommendations

Despite a legacy of debate and multiple interpretations, the diversity of rival population concepts can also be seen as a testament to the importance of understanding, recognizing, maintaining and protecting population level biodiversity and evolutionary processes within species (Moritz, 2002). However, determining the significance of a population unit is not strictly a theoretical or objective scientific exercise. Observed patterns and processes of population structure should consider how evolutionary or ecological concepts relate to the conservation or management context for the species. Generally, studies of odontocetes were applied, assumed an inclusive definition of significant population structure, with more than half making recommendations regarding the management of population units (Table 2.3). While it may not be possible or even desired to converge on an agreed definition for the evolutionarily significant unit (ESU), researchers could improve interpretation by explicitly defining population structure and the criteria used to assess and delimit populations. The impact and interpretation of studies could also be improved if studies related their work to the jurisdictionally relevant terminology, and where possible be more specific about evidence-based management recommendations.

As species management questions provide both a mandate and funding for research, it should be emphasized that even theoretical studies can have practical applications for managers, and sometimes significant implications for species. In the papers reviewed, researchers invoked a diversity of theoretical patterns and processes potentially responsible for the structure of cetacean populations. However, pattern-process explanations were sometimes conflated, vaguely described or altogether missing, challenging understanding of mechanisms, ecological or evolutionary significance, threats for management and further synthesis. Further consideration for how hypothetical structure and observations align with theoretical allopatric, parapatric or sympatric patterns, while also distinguishing potential processes or drivers from resulting patterns, would support broader understanding of trends across taxa and within the field of population biology.

2.7 Future Research Directions

Over the last 30 years the history of research on cetacean population structure has grown substantially, influencing the conservation and management of cetacean populations through the effort invested in hundreds of studies. It is a considerable accomplishment given the challenges, however there are still critical gaps in our understanding of cetacean population structure.

After an in-depth review of odontocetes, similar questions on the nature and extent of population structure in mysticetes remain to be answered. Phylogenetic trends may exist within the families of cetaceans, and would be useful to understand. With more balanced data across all possible species and with careful consideration of differences in study design, terms and scale, future metanalysis could broaden our understanding of biological and ecological patterns of population structure across Cetacea. Another interesting area of research relates to identifying natural scales of population structure, and multi-scale analyses in general. A hierarchical or nested scale framework could be used to assess unit boundaries, detect spatial autocorrelation in significance and the extent of structuring processes in the marine environment.

Future research could also improve our appreciation for how demographic events, such as historical whaling, may have led to extinctions of ESU's and affected the evolution of contemporary population structure. Understanding how human impacts have changed patterns and processes of population structure for cetaceans is relevant for currently declining species and cetaceans threatened by future impacts (e.g., range shifts) associated with climate change.

2.8 Conclusions

1. This review has covered the development of cetacean population structure studies, which have expanded in scope and complexity following the end of commercial whaling. Moving on from questions of stocks and sustainable yield to concerns for the preservation of biodiversity, the contemporary focus has been on managing evolutionarily or ecologically significant units.
2. The field has grown to adopt and apply new methods to address the questions at hand, with rapidly advancing genetic techniques proving to be the backbone of analyses. While other methods, such as acoustics or stable isotopes, are likely more appropriate for detecting ecological or cultural populations, sympatric population structures in cetaceans have emerged as both a theoretical and applied challenge for species management to reconcile.
3. Ranging from regional to global scale resolution, research has also increased in scope, building capacity and understanding with important applications for the protection and management of species. For cetaceans recognized to be most at risk,

studies of their population structure have been instrumental in detecting cryptic species or sub-species (e.g., *Sousa chinensis, Cephalorhynchus hectori, Neophocaena phocaenoides*); as well as drawing widespread awareness of their vulnerable status and emphasizing cautionary management.

4. Despite hundreds of studies on cetacean population structure, there are still large knowledge gaps in the literature across a number of jurisdictions, habitats and species. While these gaps disproportionately affect a number of cetaceans that are currently under threat or data deficient, they also represent opportunities for future research.
5. The implications of studies for species management, strongly suggests that the science-policy interface is an inevitability for cetacean population structure research that should not be ignored. To avoid misinterpretation and increase impact, research should take into account existing conservation and management paradigms.
6. Ultimately both evolutionarily and ecologically significant population structure are likely critical for species conservation (Taylor & Dizon, 1999), given the range of scales, patterns and processes, research in cetacean population structure has the potential to make significant contributions to understanding and protecting our global biodiversity.

Chapter 3 Evolutionary Impacts Differ Between Two Exploited Populations of Northern Bottlenose Whale (*Hyperoodon ampullatus*)

3.1 Publication Status

This chapter was published in Ecology and Evolution in November 2019. Feyrer, L. J., Bentzen, P., Whitehead, H., Paterson, I. G., & Einfeldt, A. (2019). Evolutionary impacts differ between two exploited populations of northern bottlenose whale (*Hyperoodon ampullatus*). *Ecology and Evolution*, *9*(23), 13567–13584.

3.2 Abstract

Interpretation of conservation status should be informed by an appreciation of genetic diversity, past demography, and overall trends in population size, which contribute to a species' evolutionary potential and resilience to genetic risks. Low genetic diversity can be symptomatic of rapid demographic declines and impose genetic risks to populations but can also be maintained by natural processes. The northern bottlenose whale *Hyperoodon ampullatus* has the lowest known mitochondrial diversity of any cetacean and was intensely whaled in the Northwest Atlantic over the last century, but whether exploitation imposed genetic risks that could limit recovery is unknown. We sequenced full mitogenomes and genotyped 37 novel microsatellites for 128 individuals from known areas of abundance in the Scotian Shelf, Northern and Southern Labrador, Davis Strait, and Iceland, and a newly discovered group off Newfoundland. Despite low diversity and shared haplotypes across all regions, both markers supported the Endangered Scotian Shelf population as distinct from the combined northern regions. The genetic affinity of Newfoundland was uncertain, suggesting an area of mixing with no clear population distinction for the region. Demographic reconstruction using mitogenomes suggests that the northern region underwent population expansion following the last glacial maximum, but for the peripheral Scotian Shelf population, a stable demographic trend was followed by a drastic decline over a temporal scale consistent with increasing human activity in the

Northwest Atlantic. Low connectivity between the Scotian Shelf and the rest of the Atlantic likely compounded the impact of intensive whaling for this species, potentially imposing genetic risks affecting recovery of this population. We highlight how the combination of historical environmental conditions and modern exploitation of this species has had very different evolutionary impacts on structured populations of northern bottlenose whales across the western North Atlantic.

3.3 Introduction

Loss of genetic diversity can threaten the persistence of populations and species by reducing individual fitness (Amos & Balmford, 2001) and limiting their potential to adapt to environmental and ecological changes (Bürger & Lynch, 1995; Lacy, 1997; Willi et al., 2006). Where species have been subjected to intensive harvesting, experienced rapid demographic decline or habitat fragmentation due to human activity, extremely low levels of genetic diversity can be an indicator of impaired recovery (Hutchings et al., 2012) and increased risk of extinction (Keller & Waller, 2002; Frankham, 2005, Frankham, 2015). However, populations that have not been through a recent bottleneck can also maintain low levels of genetic diversity through natural processes, such as climate regime shifts (De Bruyn et al., 2009; Attard et al., 2015; Westbury et al., 2019), life history attributes (Romiguier et al., 2014), social structure (Whitehead, 1998), recurrent selective sweeps (Bazin et al., 2006), and sexual selection (Amos & Harwood, 1998). Populations that have maintained low genetic diversity under equilibrium conditions are unlikely to harbour the same frequency of deleterious alleles as populations that have undergone recent genetic bottlenecks (Keller & Waller, 2002). Determining the cause of low genetic diversity in a population is crucial to understanding the genetic risks faced by species that have been subject to historical or ongoing anthropogenic impacts and informing management decisions that could determine their future persistence (Allendorf, 2017).

The northern bottlenose whale (*Hyperoodon ampullatus,* Figure 3.1) currently has the lowest known mitochondrial diversity of any cetacean species (Whitehead et al., 2017), but it is unknown whether this is due to recent declines from human harvesting or natural processes acting over longer time scales. *H. ampullatus* was severely exploited over the course of the 19-20th centuries, and its current population size and recovery status is poorly understood.

Figure 3.10 Photo of northern bottlenose whale. (*H. ampullatus*) spy-hopping in the Gully, Scotian Shelf

The range of *H. ampullatus* is restricted to the cold-temperate North Atlantic, where approximately two-thirds of the pre-whaling population estimate of 65,000 -100,000 whales were commercially whaled (Whitehead & Hooker, 2012). This level of exploitation likely resulted in a severe population decline (Christensen, 1973). Most of the early whaling effort was focused in core areas in the eastern north Atlantic, but in later years as catches declined whalers moved west, until commercial whaling of the species ended in 1971. Catch distributions suggest core whaling areas may reflect population structure, with subdivisions between the Scotian Shelf, the Labrador Sea, Iceland, Norway, and Svalbard (Whitehead & Hooker, 2012; Benjaminsen, 1972). If severe exploitation of *H. ampullatus* imposed a genetic bottleneck, population genetic theory predicts that deleterious alleles could increase in frequency, negatively impacting the recovery of their populations, especially where connectivity between core areas of abundance may be low (O'Grady et al., 2006; Keller & Waller, 2002).

While we know little about the other populations of northern bottlenose whales, the Scotian Shelf population has been the subject of long-term field study, ongoing monitoring, and is currently listed as Endangered under Canada's *Species at Risk Act* due to small population size and isolation (Whitehead et al., 1997; O'Brien & Whitehead, 2013; COSEWIC, 2011). This population is centered in the Gully, a submarine canyon and marine protected area (MPA). Between 1962-1971, commercial whaling took 87 whales from the Gully, and more than 800 whales from the nearest known population off Northern Labrador. Reduced catch per unit effort across the North Atlantic suggests northern bottlenose whale populations were depleted when whaling ended in Canada in 1971 (Christensen, 1975; Mitchell, 1977; Reeves et al., 1993; Whitehead & Hooker, 2012). Despite almost 50 years for population recovery, the most recent estimate from long-term mark-recapture studies in the Gully indicates the Scotian Shelf population has remained small and stable at ~143 (CI = 129 to 156) from 1988-2011 (O'Brien & Whitehead, 2013). It is uncertain whether recovery has been limited by genetic, demographic, or ongoing anthropogenic factors (Whitehead & Hooker, 2012). Previous genetic analyses of 10 microsatellites and mitochondrial DNA control region sequences found genetic subdivision between the Scotian Shelf and the Northern Labrador Sea regions, but no evidence of genetic bottlenecks (Dalebout et al., 2001; Dalebout et al., 2006). However, the extremely low genetic diversity detected in *H. ampullatus* by Dalebout et al. (2006), particularly at mitochondrial control region sequences (5 haplotypes differentiated by 4 polymorphic sites in 127 individuals), provided limited power to resolve recent or historical demographic changes. Whether exploitation has contributed to the extraordinarily low genetic diversity or the population structure of *H. ampullatus* is therefore unclear.

There are a number of life history and selective processes that can sustain low levels of intraspecific genetic diversity over evolutionary time scales, predating major commercial exploitation efforts (Carroll et al., 2018, Vachon et al., 2018; Ellegren & Galtier 2016; Attard et al., 2015). However, the patterns seen in *H. ampullatus* are not well explained by life history attributes commonly associated with naturally low genetic variation. Large body sizes, long generation times and lifespans have been associated with low genetic diversity in animals (Romiguier et al., 2014). Although *H. ampullatus* can grow larger than the average length found across all species of cetaceans, their body size is less than a third of the largest cetacean species, and their generation time is average compared to empirical and modelled estimates of age at first reproduction across cetacean species (Taylor et al., 2007; Christensen, 1973). Sexual selection can lead to higher variance in reproductive success among males, which has been shown to reduce nuclear diversity relative to neutral expectations (Ellegren & Galtier, 2016; Wilson Sayres, 2018). Male sexual dimorphism is prevalent across Ziphiidae, and sexual selection has been widely accepted as an explanation for why they are the most diverse family of cetaceans (Dalebout et al., 2008; Gol'din, 2014). Sexual selection has been widely accepted as an explanation for why they are the most diverse family of cetaceans (Dalebout et al., 2008; Gol'din, 2014), but this does not explain the low diversity within *H. ampullatus* relative to other Ziphiidae. Cultural selection has been identified as a mechanism that can reduce mitochondrial diversity in matrilineal whales through cultural-hitchhiking (Whitehead 1998; Whitehead et al., 2017), and though *H. ampullatus* live in social groups, they form short term “fission-fusion” associations rather than long term matrilineal structures associated with cultural hitch-hiking (Gowans, Whitehead, & Hooker, 2001). Recurrent selective sweeps for adaptive traits can reduce genetic diversity at mitochondrial loci (Bazin et al., 2006; Morin et al., 2018) and have previously been considered as a potential cause of low diversity in sperm whales (*Physeter macrocephalus,* Morin et al., 2018), killer whales (*Orcinus orca*, Foote et al., 2016) and false killer whales (*Pseudorca crassidens*, Martien et al., 2014). Some authors have suggested that deep diving, a trait shared across Ziphiidae, may be an adaptation that is under positive selection in cetaceans, however other beaked whale species, *Ziphius cavirostris* (Dalebout et al., 2005), and *Mesoplodon mirus* (Thompson et al., 2016), do not appear to share the same low diversity as *H. ampullatus*. Our current understanding of the biology of *H. ampullatus* is limited and does not provide a clear explanation for low observed diversity.

Species with smaller population sizes are expected to have lower genetic diversity due to increased genetic drift (Leffler et al., 2012), and while population sizes are poorly understood in cetaceans, current population estimates for *H. ampullatus* are larger than at least five other species of cetaceans with higher measures of range-wide mitochondrial nucleotide diversity (Vachon et al., 2018; IUCN, 2018). Historical demographic fluctuations can have lasting impacts on genetic diversity, and population dynamics associated with glacial oscillations are known to have had a major influence on patterns of genetic diversity in sub-polar and temperate species (Hewitt, 2000). During the last glacial maximum (LGM) ~19-26 kya, the sea level was lower and ice sheets expanded towards the equator, resulting in genetic bottlenecks for many species including cetaceans in the northern (Moura et al., 2014; Phillips et al., 2013; Jenkins et al., 2018) and southern hemispheres (Attard et al., 2015). In the North Atlantic, ice sheets covered a large portion of the current distribution of *H. ampullatus* (Paul & Schäfer-Neth, 2003). This likely reduced available habitat for *H. ampullatus*, possibly limiting their population size and shifting their range southwards, followed by a population expansion as available habitat increased upon glacial recession. The last glacial maximum may have disproportionately affected *H. ampullatus* relative to other cetacean species due to a large portion of their shelf edge habitat being inaccessible and their specialization for deep water prey, primarily squid from the genus *Gonatus* (Hooker et al., 2001). Climatic fluctuations, foraging preferences, and a limited polar distribution relative to other species of beaked whales may have reduced available habitat and constrained overall population size, contributing to the low mitochondrial genetic diversity currently found in *H. ampullatus*.

Here, we investigate whether the extremely low mitochondrial diversity in the northern bottlenose whale results from genetic bottlenecks associated with intensive whaling or historical demographic changes during the last glacial maximum. Using a large panel of newly developed microsatellite markers and whole mitochondrial genomes, we first resolve population structure sampled across the Scotian Shelf, Labrador Sea, Davis Strait, Iceland, and Newfoundland. The specimens from Newfoundland represent the first observations of *H. ampullatus* in an area between the two known population centers of the Scotian Shelf and Labrador-Davis Strait, a region that has not, to our knowledge, been previously described in whaling records or scientific surveys. We examine the origin of the Newfoundland whales to assess whether they may represent an unexploited population, mixing between previously described subdivided populations, or signify the potential recovery and expansion of one of the core populations. We use whole mitochondrial genomes to reconstruct the historical demography of *H. ampullatus* and assess whether there is evidence of recent or historical genetic bottlenecks in the evolutionary trajectories of sub-populations. This represents the first population genetics study of northern bottlenose whales using mitogenomes and a large number of microsatellites from contemporary samples collected across the western North Atlantic.

3.4 Materials and Methods

3.4.1 Sample Collection

Initial samples were collected from 167 northern bottlenose whales (77 females, 90 males) from six locations in the North Atlantic: the Davis Strait, Northern Labrador Sea, Southern Labrador Sea, northern Iceland, the Scotian Shelf and Newfoundland (Figure 3.2). Tissues sampled included dried gum tissue scraped from archived teeth collected during whaling more than 45 years ago (1967-1971), from biopsy samples collected 1997-2018 and samples collected from stranded whales around the region between 1994-

2005. The sampling protocol for biopsy collection was reviewed and approved by the Dalhousie University Committee on Laboratory Animals and collected under permit from Canada's Department of Fisheries and Oceans (DFO). This study considers additional samples from the Scotian Shelf, a new sampling region in Newfoundland, and contemporary samples from the Davis Strait, that have not been included in any previous population analyses.

Northern Labrador samples were obtained from dried gum tissue collected from the teeth of 80 whales killed by whalers in the Northern Labrador Sea in 1971 (Christensen, 1973). Ten Davis Strait biopsy samples were collected opportunistically during Department of Fisheries and Oceans research cruises in 2017 and 2018. Three biopsy tissue samples collected in Southern Labrador in 2003 by Dalebout et al. (2006) were reanalyzed. For North Iceland, dried gum tissue was obtained from seven whales killed in the Norwegian hunt in 1967 (Benjaminsen, 1972). Gum tissue from whaling samples was collected as per Dalebout et al. (2006). For the Scotian Shelf samples, 60 biopsies of 54 unique individuals were obtained from free-swimming whales in the summers of 1996, 1997, 2002, 2003, 2013, 2015, 2016, and 2017 using a crossbow biopsy system as per Hooker et al. (2001). Biopsies were similarly collected from 12 whales in an area off Newfoundland, which was discovered during acoustic surveys of the continental slope in 2015 (Feyrer, unpublished data, Figure 1.2), and was revisited in 2016-2017. This region has not to our knowledge been previously described as having a significant abundance of northern bottlenose whales, and no bottlenose whales were seen or sampled between the reported areas (Figure 3.2). Five additional tissue samples were collected during necropsies from northern bottlenose whales that stranded around Atlantic Canada between 1994-2005, and were provided by the Newfoundland, Maritimes and Quebec regions of DFO. Biopsy and stranding samples were preserved in 20% salt-saturated dimethyl-sulphoxide (DMSO) or 70% ethanol and stored at 4 °C or −20 °C prior to genetic analysis.

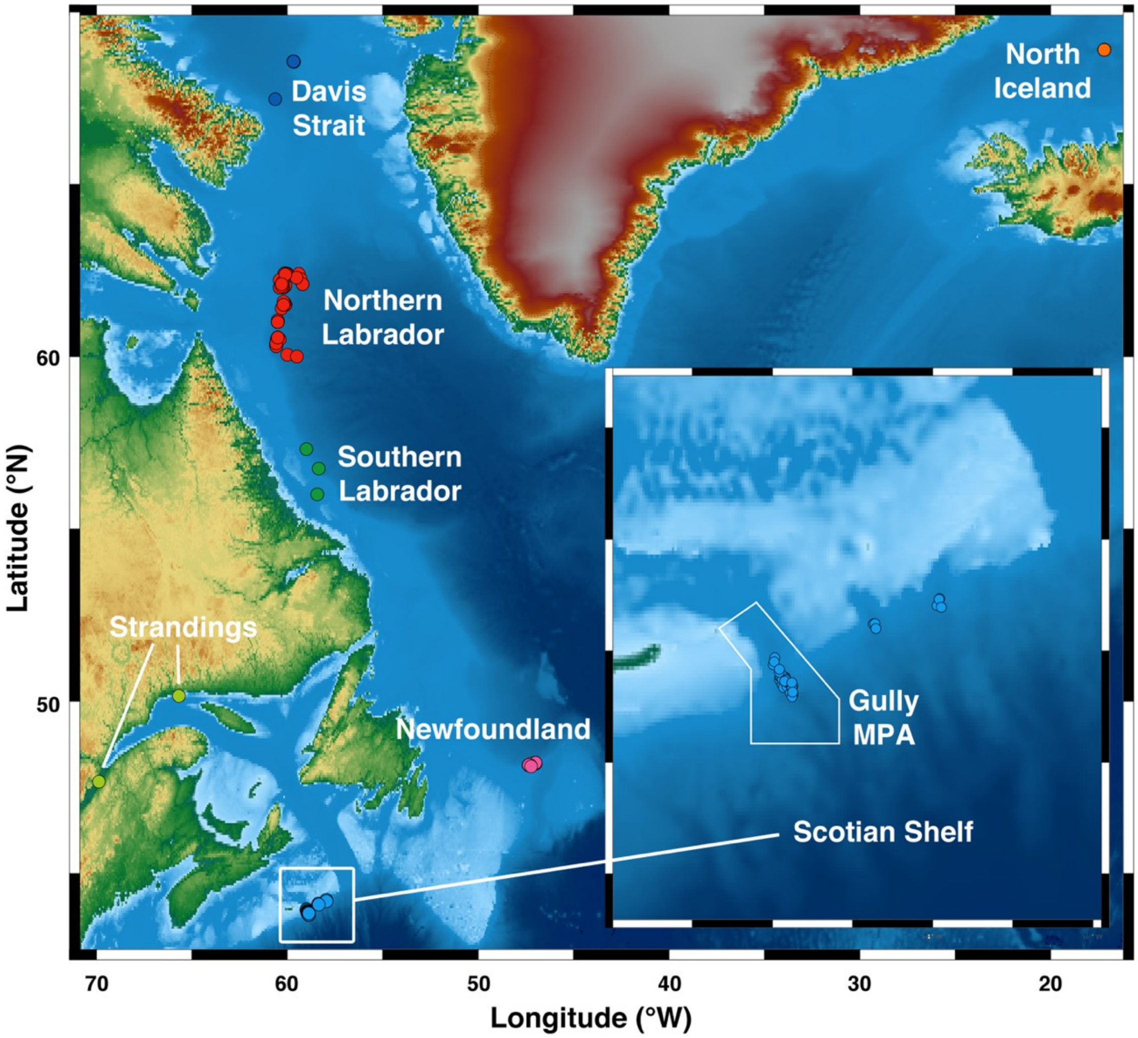


Figure 3.11 Location map of successfully sequenced samples across the study area, with inset of the Scotian Shelf. Circles indicate a sample colors indicate population: Red – Northern Labrador, green – Southern Labrador, pink – Newfoundland, orange – Iceland

3.4.2 DNA Extraction

DNA extraction was performed with a glass-binding/filtration protocol (Elphinstone et al., 2003) on alcohol or DMSO preserved fresh tissues. For archived gum tissue scrapings, DNA extraction was performed using a standard phenol/chloroform protocol (Sambrook et al., 1989).

3.4.3 Microsatellite Development, and Genotyping

Genomic DNA sequences were obtained from two *Hyperoodon ampullatus* collected in 2016, one from Newfoundland and one from the Scotian Shelf. DNAs were extracted using a standard phenol, chloroform, isoamyl-alcohol technique (Sambrook et al., 1989) from biopsied skin tissue. DNAs were sheared using a Covaris M220 Focussed-ultrasonicator following the manufacturer's protocols. We used 1µg input DNA per sample, and a target peak of 350 bases in a sample volume of 130 µl (Covaris PN 520045). Covaris settings included 42s duration, peak power 70, duty factor 20 and cycles per burst 1000. The sheared library was prepared for sequencing by strict adherence to the Illumina Truseq protocol. The prepared library was enriched via PCR amplification following the Ilumina Truseq protocols and sequenced using an Illumina Miseq Reagent Kit v2 (500-cycles).

We used MSATCOMMANDER (Faircloth, 2008) to identify microsatellites containing sequences and design PCR primers. Search criteria included a minimum of 10 repeats for tri-nucleotide motifs and a minimum of 20 for di-nucleotide repeats with target amplicon lengths between 70 and 130 bp. This amplicon size allows us to sequence the PCR products using Illumina MiSeq Reagent v3 (150-cycle) kits in a single 150 base read length. While longer reads lengths are possible, the 150-base read length was cost effective, so we designed amplicons with this in mind.

We tested 96 loci: 87 newly developed loci and 8 "legacy" loci previously used by Dalebout et al. (2006), and one sex-determining locus (CET-SEX; Konrad et al., 2017). Loci were initially vetted versus four samples in three PCRs each containing 32 multiplexed loci per sample using Qiagen Multiplex PCR kit reagent. Microsatellite alleles were evaluated following allele calling with the software MEGASAT (Zahn et al., 2017, see below). Loci were dropped if they failed to amplify, amplified more than two alleles or had evidence of null alleles. We retained 58 loci for further investigation. Of the eight legacy loci (see Dalebout et al., 2006), we dropped six for failing the criteria listed above. It is worth noting that 'legacy' loci were originally designed for imaging on electrophoretic systems, generally with an amplicon size that exceeds 150 base read length. While it is not always possible to design new priming sites to convert a legacy locus to an NGS locus, it can sometimes be done with additional investment, however here we considered 58 loci sufficient. We ran these loci versus a larger set of samples (n=153) to further evaluate their performance, dropping loci which proved invariant or amplified unreliably. We retained 49 loci for data collection and analyses (Supplementary Data, Feyrer et al., 2019a).

3.4.3.1 Library Preparation and Allele Scoring

Sequencing libraries were prepared using two sequential PCRs. Microsatellite-specific oligonucleotides were designed with a 5' tail specific to the Illumina sequencing primers. That is, each left-microsatellite-specific-oligo was tailed: CCCTACACGACGCTCTTCCG ATCT and each right-microsatellite-specific oligo was tailed: GTTCAGACGTGTGCTCTT CCGATCT. These oligos were used in multiplex-PCRs to amplify the specific microsatellite loci. Multiplex PCRs were pooled per sample, diluted 10-fold in water and used as template for the index PCR. The oligonucleotides in the index-PCR prime from the tail sequences above, and include a unique 6-base 'index' sequence plus the Illumina adapter sequence which allows the resulting DNA fragment to bind to the flow cell in the sequence step. Thus, the index-PCR step adds unique index (= barcode) sequences, allowing each individual to be demultiplexed from the sequence output, the only limitation being the number of unique index combinations available and the desired depth of sequencing per sample. Following the index PCR, samples were pooled into libraries (1μl of PCR product per sample) for sequencing. The resulting libraries were sequenced using Illumina MiSeq Reagent Kit v3 (150-cycle). Libraries were loaded with a target depth of 500 reads per sample per locus. We chose this target as a trade-off between exceeding a minimum depth threshold of 50 reads (per sample per locus) in the weakly amplifying loci versus total sequencing cost-per-sample. We used MEGASAT (Zahn et al., 2017) with default allele-calling parameters, an allowable mismatch of 2 and a minimum depth threshold of 50 reads.

MEGASAT output histograms were examined to verify the allele calls and problem loci were manually edited when necessary.

3.4.4 Mitogenome Sequencing and Assembly

We prepared a genomic library for each sample by shearing the extracted DNA, attaching sequencing adapters to the DNA fragments, and shotgun sequencing following the methods of Therkildsen & Palumbi (2017). Preliminary sequencing was performed on an Illumina MiSeq at Dalhousie University. Once the sequencing approach was optimized, we conducted additional sequencing using an Illumina HiSeq platform at Genome Québec.

We used a custom pipeline in R (R Core Team, 2018) to isolate mtDNA fragments for each individual and assemble them into complete mitogenomes. We simultaneously trimmed Illumina adapter sequences from all reads and applied stringent thresholds for sequence quality at leading and trailing bases (Q-score > 25) and over a sliding window of 4 bases (average Q-score > 23) using Trimmomatic v0.38 (Bolger et al., 2014). We mapped reads from each sample to an *H. ampullatus* reference sequence (GenBank Accession: NC_005273_1) using the Bowtie2 v2.3.4.2 aligner (Langmead & Salzberg, 2012). We then performed an iterative assembly process with MIRA v4.0.2 (Chevreux et al., 1999), first creating a draft guided assembly for each sample using the *H. ampullatus* reference mitogenome and calculating intermediate statistics of assembly performance. Because guided assembly can lead to miscalled insertion and deletion variants, we then performed *de novo* draft assembly with MIRA for samples that passed an initial completeness threshold of 95%. For samples passing guided assembly but failing *de novo* assembly, the guided draft assembly was passed to the next step in the pipeline and manually inspected for errors at the end of the assembly process. To account for overhanging genome ends that result from assemblers treating circular mitochondrial genomes as linear, we split draft assemblies at the beginning of the mitochondrial control region and merged these sequences based on their overlap, creating draft mitochondrial genomes of consistent length. To identify errors in each assembly, we re-mapped reads from each sample to the corresponding assembly with Bowtie2 and used Pilon v1.22 (Walker et al., 2014) to correct miscalled bases, fill gaps, and identify ambiguous bases using read-based evidence.

3.4.5 Genetic Variation

3.4.5.1 Microsatellites

We calculated indices of genetic variation for each regional group and overall using Hierfstat v0.04.26 (Goudet, 2005), including number of alleles, Simpson's index of allelic diversity, H_o, H_e, and inbreeding coefficients (F_{IS}). We tested for linkage equilibrium (Agapow & Burt, 2001) amongst microsatellite loci using Poppr v2.8.0 (Kamvar et al., 2015). We tested for deviations from Hardy-Weinberg Equilibrium (HWE) for each locus by population and overall using Pegas v0.10 (Paradis, 2010).

3.4.5.2 Relatedness

To assess whether kinship can be evaluated using microsatellite data, we estimated Wang's coefficient of relatedness between all pairs of individuals with 95% confidence intervals and maximum likelihood estimates of inbreeding within populations using Related v1.0 (Pew et al., 2015).

3.4.5.3 Mitogenomes

After removing technical replicates and duplicates from multiple encounters of the same individuals, we calculated nucleotide diversity, private haplotypes, and Tajima's D using Pegas v0.10 (Paradis, 2010) and custom functions for each group and overall. We constructed a phylogenetic tree based on Kimura 2-parameter distances (Kimura, 1980) using a neighbor-joining algorithm in Ape v5.0 (Paradis et al., 2004), rooted with the outgroup *Ziphius cavirostris* (Morin et al., 2013; GenBank accession: KC776706.1). We evaluated node support by performing 1000 bootstrap replicates. We inferred an unrooted haplotype network using a median-joining algorithm (Templeton et al., 1992) implemented in Pegas v0.10.

3.4.6 Population Structure

3.4.6.1 Genetic differentiation among stratified samples

To determine whether sampled regions were genetically differentiated while accounting for temporal variation in sampling time, we performed analyses of molecular variance (AMOVAs) with samples stratified by region and year collected using Ade4 v1.7-13 (Dray & Dufour, 2007). We tested for significant differentiation using 1000 permutations based on genetic distance from allele frequencies (F_{ST}) for microsatellites, and Kimura 2-parameter corrected distances (Φ_{ST}) for mitogenomes (Excoffier et al., 1992).

3.4.6.2 Bayesian clustering

To infer the number of genetic clusters in northern bottlenose whales and their spatio-temporal distributions, we performed Bayesian clustering of microsatellite genotypes using Structure v2.3.4 (Pritchard et al., 2000; Falush et al., 2007). We used an admixture model with correlated allele frequencies to allow for mixed ancestry of individuals between genetic clusters. To account for differences in sample sizes and the expectation that both sampling location and year may be informative about ancestry, we used location-year groups as priors (Hubisz et al., 2009; Wang, 2017). We averaged model log-likelihoods and individual assignment coefficients over 10 runs of 100,000 steps following a burn-in of 100,000 steps for each value of k from 1 to 5, and determined the best value of k using the ΔK method (Evanno et al., 2005).

3.4.6.3 Sex-biased dispersal

To assess whether sex-biased dispersal influences population structure, we conducted Bayesian clustering separately for each sex with Structure v2.3.4, and compared distributions of the estimated association between loci (i.e. linkage disequilibrium estimate r_d; Agapow & Burt, 2001) using Poppr v2.8.0 (Kamvar et al., 2015).

3.4.6.4 Assignment tests

To determine whether individuals recently sampled in Newfoundland, Southern Labrador, the Davis Strait, or from strandings, represent migrants from Northern Labrador or Scotian Shelf, we conducted individual assignment based on reporting groups using a Bayesian approach with Rubias (Anderson & Moran, 2018). We defined Northern Labrador and Scotian Shelf regions as reporting groups based on results of Dalebout et al. (2006), and assessed the accuracy of self-assignment to these groups as the proportion of correctly assigned individuals using a leave-one-out (Anderson et al., 2008). We explicitly tested whether individuals might not belong to either reporting group, using a Bayesian posterior probability of assignment to 0.70 to minimize the potential for Type I error (following Vähä et al., 2011).

3.4.7 Trends in Effective Population Size and Demographic Reconstruction

We estimated N_e using the linkage disequilibrium method of Waples and Do (2008), as implemented in NeEstimator V2.1 (Do et al., 2014). To infer the demographic histories and evolutionary trajectories of genetically distinct populations, we used mitogenomes to construct extended Bayesian skyline plots using BEAST v2.4.5 (Drummond et al., 2012; Bouckaert et al., 2014). Bayesian skyline analysis assumes panmixia amongst individuals, and we therefore analyzed the Scotian Shelf separately from all other samples based on clustering analyses from microsatellites and the regional structure identified by AMOVA for mitogenomes. We used a strict molecular clock model of 1.73×10^{-8} subs/site/year based on the mean mitochondrial substitution rate in Cetacea (Ho & Lanfear, 2010), with the population model parameter set to 0.5 to account for matrilineal inheritance of mitochondrial DNA. To determine the best evolutionary model for mitogenomes, we first ranked substitution models using bModelTest v0.3.2 (Bouckaert & Drummond, 2017) with a MCMC chain of 10,000,000 states. The best model was HKY with invariable sites (mean proportion = 0.66) and rate heterogeneity (mean shape/rates = 0.2351), which we used with rate and shape estimates as priors for all subsequent analyses. For the extended Bayesian skyline analyses, we ran a chain of 100,000,000 states, sampling every 5,000 states. We assessed convergence in each analysis by comparing posterior distributions in Tracer v1.6 and assessing the effective sample size (ESS>200) for each estimated parameter.

3.5 Results

3.5.1 DNA Extraction and Microsatellite Validation

DNA obtained from older tissues was of variable quality, DMSO preserved skin tissues tended to yield high quality DNA; whereas, yields from historical gum samples were poorer, both in terms of DNA quantity and quality.

The average number of microsatellite loci successfully genotyped was 46.4 for northern bottlenose whale samples and 34.8 for other beaked whale species. The success or failure of all microsatellite amplifications and summary statistics such as numbers and sizes of alleles, observed and expected heterozygosity for variable primer pairs that reliably amplified is included in Feyrer et al., 2019a (Table S1). Of the 58 loci tested only 37 microsatellite loci were scored unambiguously for the majority of northern bottlenose whale samples and had variation (Table S1; Feyrer et al., 2019a), with <1% missing data per region. The other 21 loci, including legacy loci, were discarded due to poor amplification or lack of variation.

Table 3.11. Genetic diversity of northern bottlenose whale, Hyperoodon ampullatus. n = number of samples, M = Male, F = Female, I = undetermined sex, π = nucleotide diversity, N = number of haplotypes, NP = number of haplotypes unique to a region. Mean number of alleles (allelic richness), Simpson's allelic diversity, Ho = observed heterozygosity, He = expected heterozygosity, alleles unique to each region.

	Mitogenomes					**Microsatellites**					
Region	***n* (M:F:I)**	***π***	**N**	**NP**	**Haplotype diversity**	***n* (M:F:I)**	**Number of alleles**	**Allelic diversity**	**Mean H_o**	**Mean H_e**	**Private alleles**
North Iceland (1967)	5 (3:2)	0.00072	5	1	1.00	7 (3:4)	2.3784	0.3546	0.3430	0.3546	1
Davis Strait (2017-18)	8 (6:1:1)	0.00114	7	3	0.96	8(6:1:1)	2.5135	0.3688	0.4005	0.3688	1
Northern Labrador (1971)	53 (33:19:1)	0.00096	36	26	0.98	67 (37:29:1)	3.1081	0.3825	0.3800	0.3825	6
Southern Labrador (2003)	3 (2:1)	0.00094	3	1	1.00	3 (2:1)	2.0270	0.3288	0.3784	0.3288	1
Newfoundland (2016-17)	10 (5:5)	0.00103	10	6	1.00	12 (7:5)	2.6486	0.3861	0.3896	0.3861	1
Scotian Shelf (1996-2016)	47 (23:24)	0.00058	15	8	0.87	54 (26:28)	2.8649	0.3837	0.3755	0.3837	4
Stranded (1994-7)	2 (2:0)	0.00000	1	1	0.00	2 (2:0)	1.9459	0.3547	0.4459	0.3547	0
All	128 (74:52:2)	0.00078	60	NA	0.97	153 (83:68:2)	3.3514	0.3912	0.3796	0.3912	NA

3.5.2 Genetic Variation

3.5.2.1 Microsatellites

Microsatellite diversity at the 37 loci included in population analyses was low, with a maximum of 8 and mean of 3.4 alleles per locus (Table S1; Feyrer et al., 2019a). Despite this low diversity, the probability of encountering an identical genotype across all 37 loci more than once by chance is 2.68×10^{-13}, indicating a high power to identify individuals by genotype. We recovered seven genotypes that were sampled twice, representing replicate samples from the same individuals. One of these was a male encountered twice during a single sampling period in 2018 in Davis Strait. The remaining six were within the Scotian Shelf region. Sample metadata and genotypes from the older instance of each resampled individual were excluded from subsequent analyses.

Two microsatellites (Hyam-108, Hyam-114) deviated from Hardy-Weinberg Equilibrium across the entire dataset ($p < 0.01$), and within Scotian Shelf ($p = 0.004$) and Northern Labrador ($p = 0.005$) regions, exhibiting homozygote excess. Across all samples, there was no evidence of linkage disequilibrium between pairs of microsatellite loci. When subdivided by region, samples from the Scotian Shelf significantly deviated from independent assortment ($r_d = 0.0114$, $p = 0.001$). This was due to a distribution of r_d with higher than expected values across all pairs of loci rather than strong association between a small number of loci, suggesting that deviation from independent assortment results from demographic processes such as inbreeding, restricted connectivity, or genetic bottlenecks, rather than physical linkage amongst microsatellite loci (Smith et al., 1993).

The mean of all comparisons for relatedness ($\bar{r}_w = -0.0304$) was normally distributed and not significantly different from zero, with the mean range of 95% confidence intervals ($\bar{r}_{w\text{-}high} - \bar{r}_{w\text{-}low} = 0.7267$) spanning values expected for both kin and unrelated pairs. While it is likely that we sampled related pairs of individuals on the Scotian Shelf, the wide distributions of relatedness estimate confidence intervals indicate that these microsatellite data have insufficient power to resolve close kin relationships amongst individuals.

Levels of microsatellite diversity were similar for each region. For each population and over all data, inbreeding coefficients (F_{IS}) did not differ significantly from zero, and maximum likelihood estimates of inbreeding did not differ significantly among populations. Simpson's diversity index ranged from 0.35 (North Iceland) to 0.39 (Newfoundland), and observed heterozygosity (H_o) ranged from 0.34 (North Iceland) to 0.40 (Davis Strait). Out of 124 alleles found across all microsatellite loci, only 14 were private or found in only one region. Most of these were found in Northern Labrador (private alleles = 6) or Scotian Shelf (private alleles = 4). All private alleles were rare (mean frequency = 0.0040; maximum frequency = 0.0131).

3.5.2.2 Mitogenomes

Whole mitochondrial genomes were successfully assembled for 128 individuals, with 110 variable sites over all samples (Feyrer et al., 2019b). The eight individuals identified as repeat samples using microsatellites were confirmed to have identical mitogenome sequences and were excluded from further analyses. Only one mutation caused a non-synonymous change in amino acid product, with alternate states coding for tyrosine or cysteine in the coding region for NADH dehydrogenase subunit 6.

Range-wide mitogenome nucleotide diversity was low ($\pi = 0.00078$). Regionally, nucleotide diversity was lowest in the Scotian Shelf ($\pi = 0.00058$), with only 15 unique mitogenome sequences recovered from 47 individuals. Northern Labrador ($n = 53$) had the most haplotypes not found in any other region ($NP = 26$) and had 60% ($N = 36/60$) of the unique mitogenome sequences found in this study. Every mitogenome sequence from Newfoundland was distinct ($n = 10$; $N = 10$), and 60% of these were not found in other regions ($NP = 6$). A bootstrapped phylogenetic tree of mitogenomes resolved several major branches within *H. ampullatus* that were represented in all sampling regions. Out of the 60 mitogenome haplotypes, 14 were found in at least two regions (Figures 3.3, 3.4). Tajima's D was only significant when considering all samples ($D = -1.88$; $P = 0.034$) and was not significant in any individual sampling region.

Figure 3.12 ***Hyperoodon ampullatus*** **mitogenome neighbor-joining tree from Kimura 2-parameter distances with bootstrap support (1000 replicates). Region, year(s) sampled and sex of each individual specified, with tip color corresponding to region. Red – Northern Labrador, green – Southern Labrador, pink – Newfoundland, orange – Iceland, light blue – Scotian Shelf, dark blue – Davis Strait, light green – Stranding.**

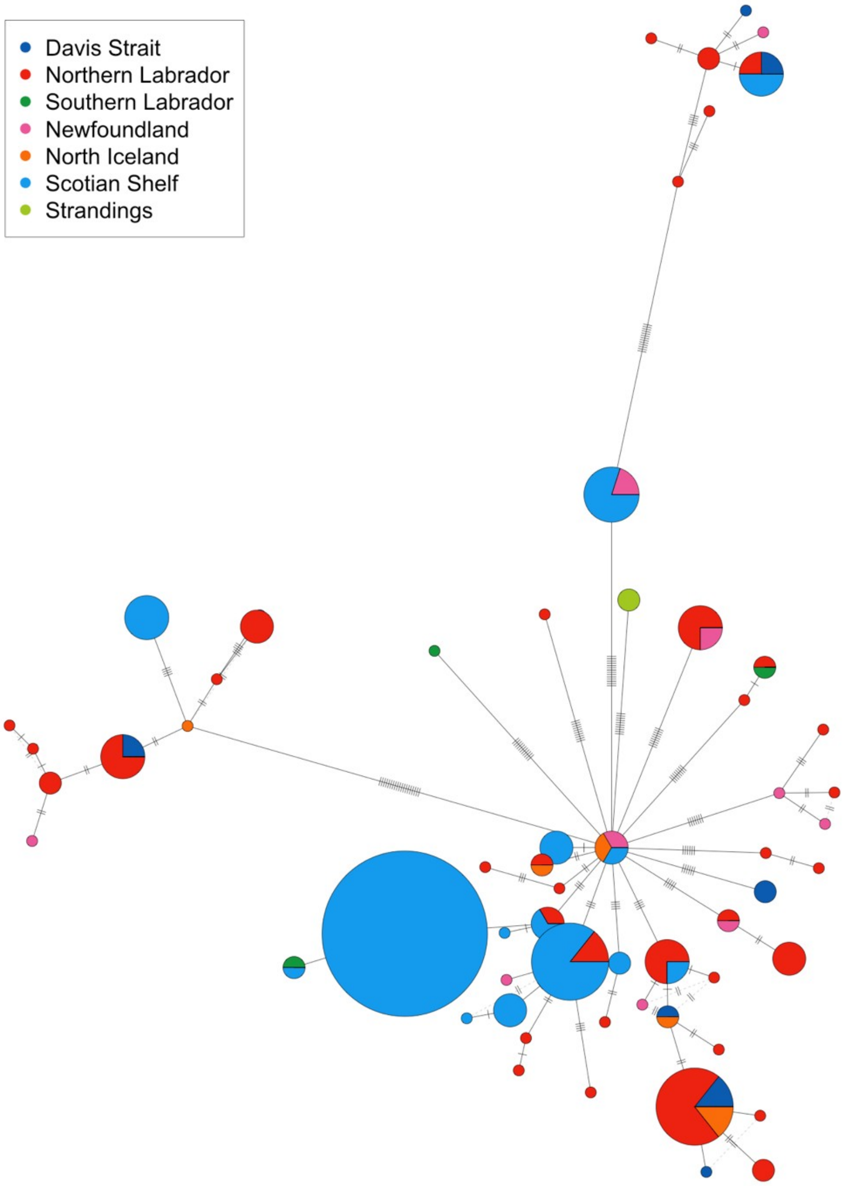


Figure 3.13 Median-joining network of *Hyperoodon ampullatus* mitogenome haplotypes. Circles represent haplotypes, colours correspond to sampling region, lines and hash marks depict number of sites differing between haplotypes, and circle area is proportional to number of samples for each haplotype. Red – Northern Labrador, green – Southern Labrador, pink – Newfoundland, orange – Iceland, light blue – Scotian Shelf, dark blue – Davis Strait, light green – Stranding.

3.5.3 Population Structure

3.5.3.1 Genetic differentiation among regions.

Analyses of molecular variance (AMOVA) revealed low but significant population structure for microsatellites ($F_{ST} = 0.013$, $P = 0.001$). Variation among regions ($\Phi_{\text{region-total}} = 0.013$, $p = 0.0010$) for microsatellites and for mitogenomes ($\mathit{\Phi}_{\text{region-total}} = 0.055$, $p = 0.015$) was higher than expected at random (Table 3.2). Pairwise comparisons of differentiation between Scotian Shelf, Northern Labrador, Southern Labrador, Newfoundland, Davis Strait, and Iceland did not identify which regions might drive population structure, with the only significant values of F_{ST} for microsatellites detected between the two regions with small sample sizes, North Iceland and Southern Labrador (modern) ($F_{\text{ST}} = 0.0707$; $P = 0.0490$) and no significant pairwise $\mathit{\Phi}_{\text{ST}}$ (based on Kimura 2-parameter distances) for mitogenomes detected in any pairwise comparisons of regions. However, AMOVA of Scotian Shelf against all other individuals grouped together showed significant regional structure for both microsatellites ($\mathit{\Phi}_{\text{region-total}} = 0.018$, $P = 0.0010$) and mitogenome data ($\mathit{\Phi}_{\text{region-total}} = 0.047$, $p = 0.02$).

Table 3.12 AMOVA results for (a) microsatellites and (b) mitogenomes between and within regions for *Hyperoodon ampullatus* in the NW Atlantic.

(a) Microsatellites

~regions (all)	**Variance proportion**		**Φ**	**HA**	**P**
Between region	1.2956	Phi-region-total	0.0130	greater	0.0010
Between samples within region	2.5027	Phi-samples-region	0.0254	greater	0.0609
Within samples	96.2017	Phi-samples-total	0.0380	less	0.0190

~regions (Scotian shelf vs. others)	Variance proportion		Φ	HA	P
Between region	1.8118	Phi-region-total	0.0181	less	0.0140
Between samples within region	2.5273	Phi-samples-region	0.0257	greater	0.0619
Within samples	95.6609	Phi-samples-total	0.0434	greater	0.0010

(b) Mitogenomes

~regions (all)	**Variance proportion**		**Φ**	**HA**	**P**
Between region	5.4829	Phi-region-total	0.0548	greater	0.0150
Between samples within region	-2.9998	Phi-samples-region	-0.0317	greater	0.2957
Within samples	97.5170	Phi-samples-total	0.0248	less	0.2468

~regions (Scotian shelf vs. others)	Variance proportion		Φ	HA	P
Between region	4.7047	Phi-region-total	0.0470	greater	0.0200
Between samples within region	-2.6363	Phi-samples-region	-0.0277	greater	0.7400
Within samples	97.9315	Phi-samples-total	0.0207	less	0.4500

3.5.3.2 *Bayesian clustering.*

To assess population structure while accounting for potential differences between both the locations and years that samples were collected in, we performed Bayesian clustering of microsatellite data in Structure with sampling units defined by location-year. The highest –log Pr(X|*k*) estimates across 10 replicate runs for each value of *k* from 1 to 5 were at *k*=2, and the ΔK method identified the highest rate of change in –log Pr(X|*k*) estimates for *k*=2. Assignment probabilities of individuals separated genetic clusters entirely by sample location rather than year (Figure 3.5, with clustering for *k* = 2 distinguishing individuals from the Scotian Shelf samples from all other individuals. Subsequent runs of only individuals from the Northern region did not detect any finer substructure within our samples. Two individuals from the Scotian Shelf (a female, NBW07-2015 and a male, HamSH96-01) had a lower assignment coefficient to the Scotian Shelf cluster (Q_{1F}=0.4728, Q_{1M}=0.3071) than for the other cluster (Q_{2F}=0.5272, Q_{2M}=0.6929), suggesting they may be, or descend from, recent migrants into the Scotian Shelf from another region. Both samples from stranded individuals in the Gulf of St. Lawrence had ambiguous clustering results and could not be reliably assigned to a source location from the STRUCTURE results.

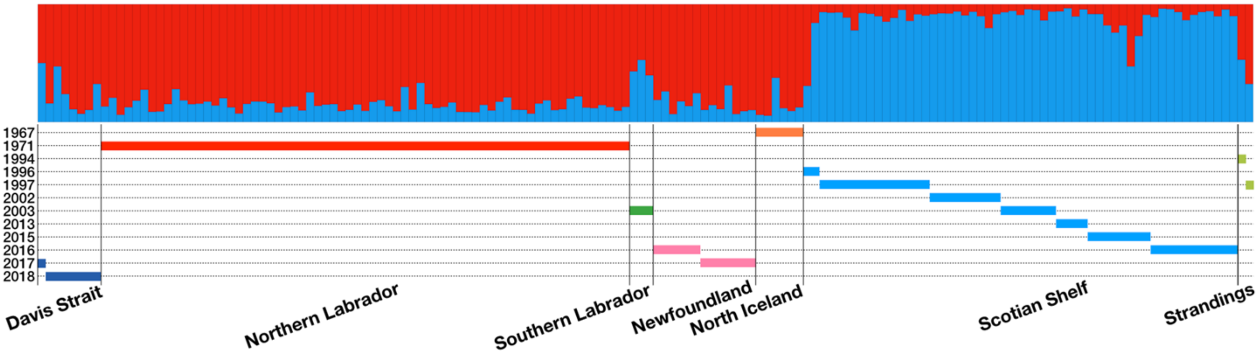


Figure 3.14 Genetic structure assigned to individual *H. ampullatus* using STRUCTURE with location-year group (n = 16) priors for k = 2. Location of sampling along the x-axis and timeline of sampling below. Red – Northern Labrador, green – Southern Labrador, pink – Newfoundland, orange – Iceland, light blue – Scotian Shelf, dark blue – Davis Strait, light green – Stranding.

3.5.3.3 *Sex-biased dispersal.*

If dispersal is unequal between sexes, the more dispersive sex is expected to have less genetic structure and lower levels of association between loci (i.e. linkage disequilibrium) than the more philopatric sex. We did not find significant differences between the distributions of estimates of linkage disequilibrium amongst microsatellites in females (r_d=0.0039; p=0.064) or males (r_d=0.0017; p=0.206). STRUCTURE assignment indices identified geographic structure between Scotian Shelf and all other regions in males but not females. This suggests that female northern bottlenose whales may be more dispersive, in contrast to the general male bias pattern of mammals (Mabry et al., 2013). However, the larger sample size for males (84) than females (70) could influence the ability to detect structure among females. The contrasting results from STRUCTURE and estimates of linkage disequilibrium suggest that the microsatellite data may not have adequate power to assess whether there is sex-biased dispersal in *H. ampullatus*.

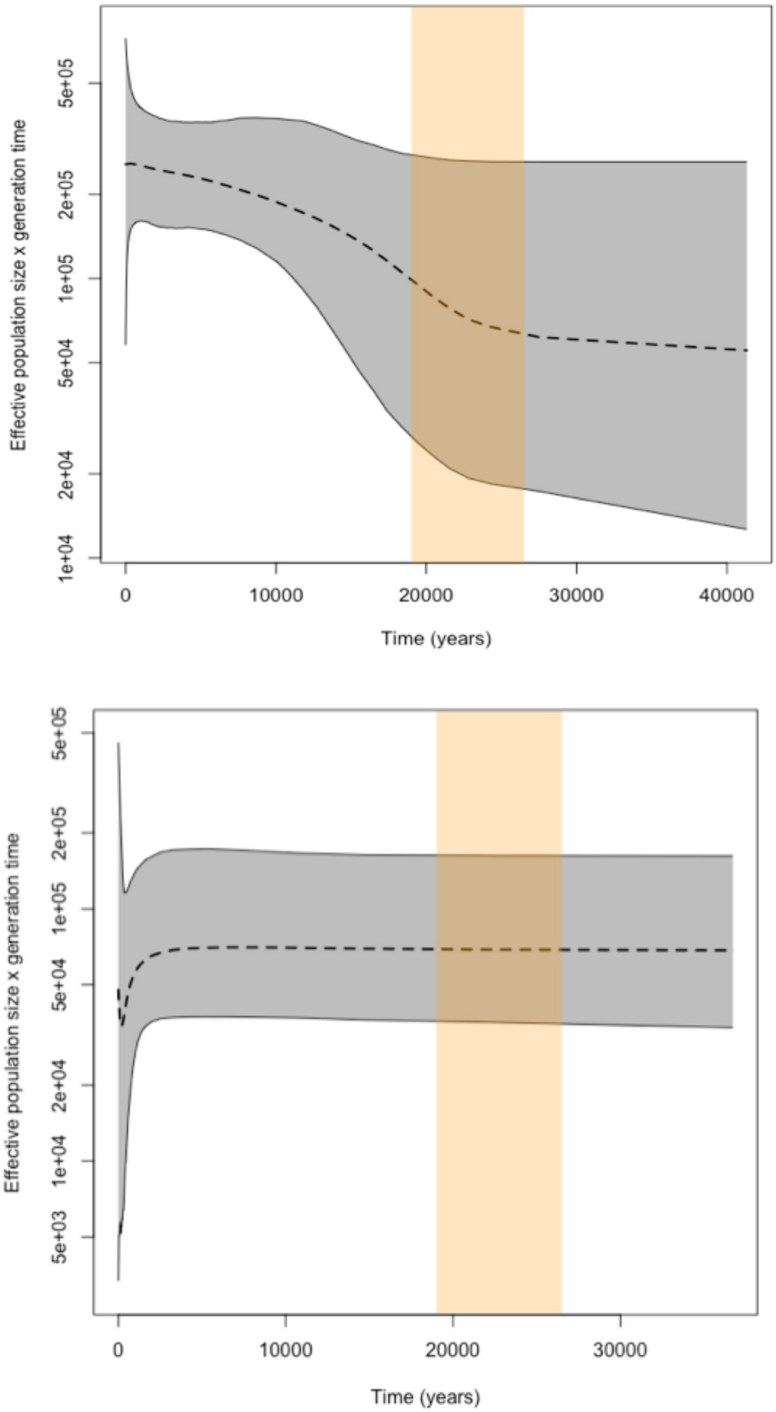
3.5.3.4 Assignment tests.

Self-assignment of individuals sampled in Scotian Shelf and Northern Labrador had 87.7% accuracy using a leave-one-out approach overall, with 85.1% accuracy in Northern Labrador and 90.9% accuracy in Scotian Shelf. Assignment of individuals sampled outside these reporting group areas revealed substantial affinity to Northern Labrador and the potential for intermediate genotypes or presence of unidentified baseline reporting units. Of the three individuals from Southern Labrador, two were assigned to Northern Labrador, and one was not assigned to a reference group. Of the 12 individuals from Newfoundland, seven were assigned to Northern Labrador, one was assigned to Scotian Shelf, and four were not assigned to a reference group. Of the eight individuals from Davis Strait, only two were assigned to Northern Labrador and six were not assigned to a reference group. Of the seven individuals from Iceland, six were assigned to Northern Labrador and one was not assigned to a reference group. Neither of the two strandings were assigned to a reference group.

3.5.4 Effective Population Size and Demographic Reconstruction

Estimates of effective population size (N_e) in each region had infinite upper bounds of 95% confidence intervals for all regions except Scotian Shelf (N_e = 54.8; 95% CI = 43.0-72.7), reflecting limited statistical power to estimate upper bounds. The lower 95% CI bounds for Northern Labrador (N_e = 495.1; 95% CI = 212.1-infinite) was higher than the upper bound for Scotian Shelf. Estimates for Newfoundland (N_e = infinite; 95% CI = 47.8-infinite), Davis Strait (N_e = infinite; 95% CI = 24.1-infinite), and Iceland (N_e = infinite; 95% CI = 18.4-infinite) overlapped with estimates for the Scotian Shelf. Due to the low statistical power caused by having only three samples from Southern Labrador, all estimates of N_e for this region were infinite. Estimates for Davis Strait, Northern Labrador, Southern Labrador, and Newfoundland combined (N_e = 1604.4; 95% CI = 409.5-infinite) were higher than for each region separately.

Demographic reconstructions differed for the Scotian Shelf population and the group consisting of all other samples (hereafter: Northern region). Because neither group is monophyletic, both are expected to have similar ranges for their time to most recent common ancestor (TMRCA). Consistent with this expectation, the estimated TMRCA for the Northern region was 47.4 kya (95% Highest Posterior Density Interval (HPDI): 32.8-61.5 kya), and the estimated TMRCA for Scotian Shelf was 46.6 kya (95% HPDI: 34.1-61.3 kya). The skyline analysis for the Northern region shows an increasing trend from the estimated time of the last glacial maximum (19.0-26.5 kya) to present times (Figure 3.6a). In contrast, the extended Bayesian skyline analysis of Scotian Shelf shows a relatively constant population size throughout the last glacial maximum, followed by a sharp decline sometime in the last two centuries (Figure 3.6b). The maximum rate of decline occurs ~350 years ago and estimates of effective population size reach a minimum value ~180 years ago. The 95% central posterior density interval reached a minimum range 360 years ago and increased since that time. While median estimates of effective population size increase after the minimum observed less than 200 years ago, the rapid increase of the 95% central posterior density intervals for years following this minimum accommodate both stable, increasing, and decreasing trends of effective population size, suggesting whole mitogenomes provide insufficient power to resolve trends in effective population size since the decline.



(a)

(b)

Figure 3.15 Extended Bayesian skyline plots for *Hyperoodon ampullatus* mitogenomes from a) Northern region; b) Scotian Shelf. Dashed line represents median reconstructed Ne, with grey shaded areas representing the 95% (Highest Posterior Density Intervals) HPDI. Beige shaded bar indicates the estimated duration of the last glacial maximum. Y-axes are

logarithmic. In the Scotian Shelf, effective population size reaches maximum rate of decline at ~350 years and minimum value at ~180 years.

3.6 Discussion

Understanding the impact of large-scale removals on species recovery and evolutionary potential ideally involves an assessment of a range of demographic, life history, and genetic correlates (Baker & Clapham 2004). Because rapid declines in genetic diversity can pose significant risks for small populations, distinguishing between naturally low levels of genetic variation and recent genetic depletion is important. There are several potential causes for low diversity in natural populations, including life history attributes, selective processes, demographic fluctuations and exploitation bottlenecks. However, for species with long histories of exploitation, such as cetaceans, pre-harvest population structure or census size is poorly known, and typically few archival specimens are available to reconstruct genetic impacts of harvesting (but see Dufresnes 2018; Phillips et al., 2013). Consequently, studies are increasingly reliant on contemporary sample-based genetic reconstructions to identify historical bottlenecks, assess genetic resilience and population recovery from past demographic events, and estimate species' evolutionary trajectories (Attard et al., 2015; Carroll et al., 2019; Emami-Khoyi et al., 2017; Foote et al., 2016). As we have outlined earlier there are a number of reasons that N_e and current census size may not be correlated, however, the uncertainty surrounding estimates of N_e and ratios applied to infer true population size (N_c) is well established (Palstra et al., 2012). N_e cannot be used as a metric to evaluate the impacts of harvesting, or the remaining evolutionary potential in a population (Palsbøll et al., 2013). Methods that consider trends in genetic diversity over time, such as Bayesian Skyline and pairwise sequentially Markovian coalescent analyses, provide a more useful historical context for interpreting currently observed patterns. Understanding conservation status should be informed by an appreciation of natural occurring diversity, past demography, and overall trends in population size, which contribute to a species' evolutionary potential and resilience to genetic risks. In the following sections we evaluate the distinctions between northern bottlenose whale populations, consider the processes that explain their demographic trends, and outline the risks associated with low genetic diversity in light of current conservation concerns for this historically harvested species.

3.6.1 Low Genetic Diversity

We detected low overall diversity in both microsatellites and mitogenomes of northern bottlenose whales, relative to other species of cetaceans based on a comparative study by Vachon et al. (2018), which accounted for differences in allelic richness between microsatellite loci, sample size, and ascertainment bias. Range-wide genetic diversity across the full mitogenome is $\pi = 0.00078$ (n = 128), the lowest found for any cetacean; the next lowest is sperm whales ($\pi = 0.00096$, $n = 175$; Morin et al., 2018). This is consistent with *H. ampullatus* having the lowest known mitochondrial D-loop nucleotide diversity across 27 species of Cetacea for which this metric is available (Whitehead et al., 2017). The reason for their low diversity is uncertain. Studies of closely related and ecologically similar species of Cuvier's and Gray's beaked whales, found higher levels of genetic diversity than *H. ampullatus* and no significant population structure within the same ocean basin, though studies were based on limited mtDNA data (290 – 590 bp) (Thompson et al., 2016; Dalebout et al., 2005). Both these species of cetaceans have a larger global distribution than *H. ampullatus*, suggesting that geographic distribution across multiple ocean basins may promote genetic diversity, which is supported by other studies that detected a relationship between mtDNA diversity and global latitudinal range (Vachon et al., 2018). Other cetaceans with low genetic diversity and geographic ranges restricted to a single ocean basin include the Narwhal (*Monodon monoceros)* and Commerson's dolphin *(Cephalorhynchus commersonii)* (Westbury et al., 2019*;* Whitehead et al., 2017). It is possible that restricted geographic distribution may be correlated with other natural factors, such as low historical population size, evolutionary specializations for prey, or environmental constraints that influence genetic diversity.

3.6.2 Importance of Population Structure

Concepts for understanding intraspecific population structure can range from demographically independent populations (DIPs), to evolutionarily significant units (ESUs), to subspecies (De Queiroz 2011; Taylor et al., 2017). Generally, ESUs are groups that are substantially reproductively isolated from other populations and embody an important aspect of the species' evolutionary potential, and may or may not be monophyletic (Taylor et al., 2017; Palsbøll et al., 2007; Moritz, 2002). In Canada, national protection for species at risk recognizes "designatable units" (DUs), which are by definition Evolutionarily Significant (COSEWIC 2015). Dispersal may occur between DUs, so long as it is insufficient to prevent local adaptation (COSEWIC 2015). The Scotian Shelf population of northern bottlenose whales has been considered a DU in Canada since 2004, due to small population size, and isolation from other populations. We detected genetic structure with microsatellite markers and mitogenomes, differentiating the Scotian Shelf population from northern areas and supporting previous work identifying the Scotian Shelf as a separate DU. While not considered here, the importance of population structure and diversity in this species requires full consideration of northern bottlenose whales across the eastern parts of their range.

Previous studies of northern bottlenose whales in the Northwest Atlantic found the Scotian Shelf was genetically distinct from Northern Labrador and Iceland, which was determined by the absence of a single mtDNA haplotype on the Scotian Shelf and significant F_{ST} between the Scotian Shelf, Northern Labrador and Iceland based on microsatellite data (Dalebout et al., 2001, 2006). With additional contemporary samples from all areas (except Iceland), we corroborated the genetic structure between the Scotian Shelf and all other regions through analyses of variation (AMOVA and Bayesian Structure) using 37 microsatellites and the full mitogenome. While all regions shared multiple mitogenome matrilines, we found unique haplotypes in each sampling area. Although excess of a few haplotypes in the Scotian Shelf appears to drive genetic subdivision between regions, due to the numerous haplotypes shared among regions it is unclear how long and to what degree the Scotian Shelf has been isolated. Based on the number of unique haplotypes found in each of the sampling locations across the northern region, it appears all areas contain significant diversity that may be important to the evolutionary potential of the species.

Management concern for the newly discovered whales sampled in the habitat off Newfoundland motivate further consideration of how these individuals fit into currently recognized population structure. The primary genetic distinction detected in our data was between the Scotian Shelf population and all other populations, which collectively formed the Northern region. The few samples from Newfoundland clustered with the Northern region in a Bayesian Structure analysis. However, additional assignment analyses of Newfoundland whales to these two reference groups suggested one grouped with the Scotian Shelf, seven with Northern Labrador, and four were not assigned. These results are not definitive on the population origin of the whales found off Newfoundland, suggesting that it may be an area of mixing between the two currently recognized DUs, and other unknown populations, or possibly represent a newly established population. Although our sample size for Newfoundland is small, due to the high proportion of unique haplotypes in this region and the low overall haplotype variation in this species, Newfoundland appears to represent a source of significant diversity.

Given few barriers in the marine environment, genetic divergence between populations may occur as the result of a number of selective pressures or low population density over evolutionary time. Recent acoustic surveys have documented northern bottlenose whales along the continental slope edge, between the Scotian Shelf and the Northern region (Chapter 1 Figure 1.2), suggesting that we may not yet have the full picture of contemporary connectivity. Ongoing genetic monitoring is required to resolve whether genetic connectivity is the result of recent historical or contemporary migration or incomplete lineage sorting from a common ancestral population. Previous studies have suggested that genetic separation of the Scotian Shelf from other regions likely pre-dates human exploitation and is not the result of a decline in population size (Dalebout et al., 2006). However, a lack of monophyletic spatial structure with mixed assignment of individuals from sampled regions outside of the Scotian shelf or Northern Labrador suggests there may be ongoing migration among regions. As discussed below, exploitation may have altered the distribution and extent of northern bottlenose whales, and related impacts to their population structure cannot yet be refuted. Given the low overall diversity in northern bottlenose whales, reducing barriers to connectivity between regions–such as those posed by offshore anthropogenic activities–is important for effective conservation with particular concern for the Endangered Scotian Shelf population.

3.6.3 Regional Differences in Evolutionary Trajectories

Few studies of cetaceans have used the full mitogenome to reconstruct demography with Bayesian skyline analyses (but see Cunha et al., 2014, Morin et al., 2013, Morin et al., 2018). Most have relied on comparatively short fragments of mtDNA (414-2494 bp) (Thompson et al., 2016; Attard et al., 2015; Phillips et al., 2013) and were not able to identify more recent impacts within the last 2-10 kya. Demographic reconstructions of other commercially whaled cetaceans, including sperm whales, pygmy blue whales, and bowhead whales, have depicted a gradual signal of population expansion and retraction temporally consistent with historical glacial cycles, but found no substantial evidence of recent declines in diversity that could be attributed to anthropogenic impacts such as whaling (Morin et al., 2018; Attard et al., 2015; Phillips et al., 2013). While the shorter DNA segments used in other studies limited power to distinguish a signal of impacts that may have occurred in the timeframe aligned with human harvesting, here we use >16,000bp of mtDNA sequenced for 128 individuals to investigate changes in effective population size at temporal scales recent enough to resolve the potential impacts of human activity.

The presence of several lineages in the mitogenome phylogenetic tree (Figure 3.3), and the star shaped expansion signal of the haplotype network (Figure 3.4), suggest that over their entire range northern bottlenose whale populations have undergone an expansion, likely following colonization of newly available ice-free habitat after the last glacial maximum (LGM). Consistent with this hypothesis, Tajima's D using all samples was -1.88 ($P < 0.05$), suggesting either a population expansion or selective sweep occurred. Population expansions following the LGM have been detected in other cetacean species in the North Atlantic (e.g. sei whales, *Balaenoptera borealis,* Huijser et al., 2018; minke whales, *Balaenoptera acutorostrata,* Anderwald et al., 2011; white-sided dolphins, *Lagenorhynchus acutus,* Banguera-Hinestroza et al., 2010).

We conducted separate demographic analyses for the Scotian Shelf and the Northern region due to the assumption of panmixia required for Bayesian Skyline analysis. Our reconstruction for the Northern region was consistent with a historical expansion following the LGM, concordant with other studies of cetaceans with temperate ranges overlapping previously glaciated habitats (Figure 3.6a). The Scotian Shelf population exhibited a more stable effective population size throughout the LGM, followed by a steep decline with a maximum slope occurring ~350 years ago and estimates of effective population size reaching a minimum value within the last 200 years (Figure 3.6b). The rapid increase in the 95% HPDI after this minimum suggests that inferences of trends in effective population since this decline are unreliable. Although an earlier study by Dalebout et al. (2006) did not find significant evidence of a bottleneck using the M-ratio and Tajima's *D*, they had significantly lower power in their analyses of 434bp mtDNA vs. the 16,450bp from the full mitogenome used here. A stable trend across the LGM is plausible, as the Scotian Shelf population exists at the southernmost edge of the species range and may have been least affected by historically colder climate regimes. However, the sudden recent decline in effective population size for the Scotian Shelf population is not consistent with major climatic oscillations. Although the precise timing of the decline within the last 200 years is highly uncertain, human activity is the only major correlate known to have occurred within this period. As whaling removed a large number of whales during this period, we infer that the effects of whaling likely had a greater genetic impact on the more isolated Scotian Shelf population than the Northern population. Below, we further consider the genetic impacts of harvesting and limitations for the recovery of this small population.

3.6.4 Characterizing Genetic Risk for Evaluating Species Recovery

Genetic risks posed by human harvesting include inbreeding depression and loss of diversity, which can present significant challenges for the recovery of small populations. However, recovery of genetically depauperate species such as cheetah (*Acinonyx jubatus;* Dobrynin et al., 2015) and the elephant seal (*Mirounga angustirostris*; Hoelzel et al., 2002) from a few breeding pairs following natural bottlenecks or human induced population declines suggests that some species may not have the same genetic load as others, potentially due to low effective population sizes maintained over evolutionary time scales by natural processes (Amos & Harwood 1998; Amos & Balmford, 2001). Even with naturally low effective population sizes, small peripheral populations are expected to be less resilient to further reductions in genetic diversity due to reduced connectivity and increased potential for inbreeding. Over a period of a few years, commercial whaling for northern bottlenose whales off Nova Scotia removed an equivalent of ~60% of the current Scotian Shelf population size. The whales in the Labrador Sea were also heavily targeted, reducing the closest known potential source of new migrants (O'Brien & Whitehead, 2013; Whitehead & Hooker, 2012). The Bayesian Skyline analysis indicates that this level of exploitation coincides with declines in the genetic diversity of the peripheral Scotian Shelf population, suggesting this small population may have an increased risk for inbreeding depression and reduced evolutionary potential to respond to a changing environment.

We were unable to adequately assess the risks associated with inbreeding depression or genetic relatedness in this study due to low overall variation across a panel of 37 microsatellite markers. Different nuclear markers may be able to better distinguish the extent of consanguinity in this small population. However, there are notably fewer mitochondrial haplotypes within the Scotian Shelf population than outside it, suggesting the dominance of a few successful matrilines within this population. Due to the very small population size (N ~143, O'Brien & Whitehead, 2013) and the low genetic diversity of whales sampled on the Scotian Shelf, there is an increased likelihood of inbreeding in this region relative to others. While we do not have empirical data on whether inbreeding depression is reducing the reproductive output and survival of Scotian Shelf northern bottlenose whales, these factors may have contributed to the slow growth and recovery from whaling observed in this population over the last 50 years (Whitehead & Hooker, 2012).

The Northern region does not appear to have suffered a recent decline in Ne. This may be due to greater connectivity between core areas, or inadequate statistical power to detect a recent decline resulting from the large proportion (46%) of whaling era samples in our analysis and low genetic diversity, which is reflected by increasing uncertainty of demographic reconstructions over the last two centuries. Comparing contemporary samples from areas in the Northern region will help validate the lack of a recent bottleneck outside the Scotian Shelf. Stable trends in the effective population size of whales in the Northern region and the new aggregation of whales observed in Newfoundland suggest that in core population centers, the species may be recovering from historical whaling. Comparisons with northern bottlenose whale populations in the eastern North Atlantic may provide additional context for these demographic trends and resolve the phylogeographic history of this species.

3.6.5 Conclusion

Low diversity in *H. ampullatus* is likely naturally occurring, but further population declines or reductions in connectivity could compromise the evolutionary potential of the species and risk the recovery of the more depleted Scotian Shelf population. The genetic risks imposed by harvesting and the slow recovery for the Scotian Shelf population identifies a number of considerations that are broadly relevant to the assessment of genetic impacts on commercially exploited species. Distinct populations can respond differently to human exploitation, and determining risk requires an assessment of range-wide population subdivision and historical trends. We highlight that understanding the evolutionary context and demographic trajectories of distinct populations, using techniques such as Bayesian skyline analysis, can reveal potential genetic risks that can help inform species conservation and management priorities. Population structure may be cryptic and require high resolution markers with the power to detect variability, particularly in species with low genetic diversity, which is important to consider when reconstructing historical demography to assess recent human impacts such as exploitation and the recovery of a species across their range.

3.7 Acknowledgements

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3.8 Data Accessibility

The data that support the findings of this study have been made openly available online through Dryad (https://doi.org/10.5061/dryad.xgxd254bx) and the pipeline for mtDNA assembly is available on Github at (https://github.com/einfeldt/Hyperoodon). DNA sequences are accessible on Genbank. GenBank accession numbers for mitogenomes are: MN536234 - MN536368, accession numbers for microsatellites are included online in Feyrer et al. 2019 (Supplementary materials).

3.9 Author Contributions

Laura Joan Feyrer conducted the field work, including biopsy collection from 2015-2017, designed the study and wrote the manuscript. Tony Einfeldt designed and conducted the genetic analyses and contributed to the interpretation of results and writing of the manuscript. Ian Paterson designed the microsatellite primers and conducted most of the laboratory analyses. Paul Bentzen contributed to the analytical design, interpretation of results and revised the manuscript. Hal Whitehead designed the study, contributed to field data collection and revised the manuscript.

Chapter 4 Prolonged Maternal Investment in Northern Bottlenose Whales Alters Our Understanding of Beaked Whale Reproductive Life History

4.1 Publication Status

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4.2 Abstract

Nursing and weaning periods are poorly understood in cetaceans due to the difficulty of assessing underwater behaviour in the wild. However, the onset and completion of weaning are critical turning points for individual development and survival, with implications for a species' life history including reproductive potential. δ^{15}N and δ^{13}C deposited in odontocete teeth annuli provide a lifetime record of diet, offering an opportunity to investigate variation and trends in fundamental biology. While available reproductive parameters for beaked whales have largely been inferred from single records of stranded or hunted animals and extrapolated across species, here we examine the weaning strategy and nursing duration in northern bottlenose whales (*Hyperoodon ampullatus*) by measuring stable isotopes deposited in dentine growth layer groups (GLGs). Using a collection of *H. ampullatus* teeth taken from whales killed during the whaling era (N = 48) and from two stranded specimens, we compared ontogenetic variation of δ^{15}N and δ^{13}C found in annual GLGs across all individuals, by sex and by region. We detected age-based trends in both δ^{15}N and δ^{13}C that are consistent across regions and males and females, and indicate that nursing is prolonged and weaning does not conclude until whales are 3-4 years old, substantially later than previous estimates of 1 year. Incorporating a prolonged period of maternal care into *H. ampullatus* life history significantly reduces their reproductive potential, with broad implications for models of beaked whale life history, energetics and the species' recovery from whaling.

4.3 Introduction

Maternal investment in mammals varies based on an array of ecological and evolutionary factors resulting in a range of maternal strategies (e.g., Rendell et al., 2019). Nursing is critical to the survival and fitness of infant mammals, providing our earliest energetic and nutritional requirements, supporting maternal bonding, and initializing ongoing socialization (Clutton-Brock, 2016; Hayssen & Orr, 2017; Whitehead & Mann, 2000). Nursing duration and the weaning strategy have implications for infant survival, interbirth interval, and lifetime reproductive output, which are critical measures for understanding the life history, energetics and population dynamics of a species (New et al., 2013). While lactation may occur over a period of weeks to years, weaning initiation and completion are important developmental turning points – as juveniles become nutritionally independent, it allows females to redirect significant energetic resources back to themselves and towards their future offspring (Lee, et al., 1991; Trivers, 1972). Weaning, which may be sudden or gradual, depends on a range of factors including the survival and vulnerability of offspring in the postpartum period, the technical difficulty of self-sufficient foraging strategies, species social structure, individual behavioural plasticity and regional prey availability (Clutton-Brock, 1989; Clutton-Brock et al., 1981; Hayssen & Orr, 2017; New et al., 2013). Responding to a range of ecological and evolutionary factors, nursing duration can vary widely among and even within species, forming the context of the weaning "conflict", with trade-offs between the fitness of offspring and future female reproductive potential (Hayssen & Orr, 2017; Trivers, 1974; Whitehead & Mann, 2000).

Maternal investment in cetaceans (dolphins and whales) is known to be extensive and provides a key role in infant survival, however our appreciation of weaning strategies is challenged by the cryptic nature of nursing behaviour and their aquatic habitat (Rendell et al., 2019). What we do know can be generalized by sub-order; with a large degree of variability among species, odontocetes appear to prolong nursing and weaning over years (mean = 21 months), while mysticetes typically wean their young within the first year (mean = 11 months) (Perrin & Reilly, 1984) (APPENDIX C C, Table C2). This difference in maternal investment has been linked to energetic resources available to income versus capital breeders (Borrell et al., 2015; Rendell et al., 2019).

Four different methods have been used to estimate nursing duration in cetaceans: stomach content analysis, cow-calf ratios, behavioural observations and stable isotope analyses, which may explain some of the discrepancies between estimates within and among species (Perrin & Reilly, 1984; Rendell et al., 2019). Across studies, behavioural observations typically reported the oldest average age at weaning (27 months), in contrast with stomach content analyses, which found average weaning age occurred much younger (16 months, APPENDIX C C, Table C2). Temporal analysis of nitrogen stable isotopes ($\delta^{15}N$) in accretionary tissues, such as sequential growth layer groups (GLGs) in dentine, have also been used to estimate weaning age and other ontogenetic shifts in individual foraging and trophic level based on nutritional physiology (Evacitas et al., 2017; Matthews & Ferguson, 2015; Mendes et al., 2007; Rossman et al., 2015). As $\delta^{15}N$ decreases during the transition from juveniles feeding exclusively on milk to independent foraging, differences in $\delta^{15}N$ between GLGs in tooth dentine can be used to estimate nursing duration and weaning completion (e.g., Matthews & Ferguson, 2015; Newsome et al., 2009). However, weaning related relationships with $\delta^{13}C$ are less clear and across studies there is no consistent trend or pattern reported for isotopic carbon found in marine mammal tissues during the dietary transition from milk to prey (e.g., Evacitas et al., 2017; Matthews & Ferguson, 2015; Newsome et al., 2010).

Due to the offshore habitat and elusive nature of deep diving beaked whales (Ziphiidae), there is a lack of baseline data on key aspects of their life history so that reproductive parameters are poorly understood (Hooker et al., 2019). Much of our understanding of their biology comes from one species, the northern bottlenose whale (*Hyperoodon ampullatus*), which was the target of a century of commercial whaling across the North Atlantic ending in the early 1970’s. In the final years of the commercial hunt in Labrador and northern Iceland, data otherwise difficult to collect today using non-lethal methods were recorded for many individuals, including age (from teeth), sex, sexual maturity, reproductive state, fetal term, and stomach contents [20]. Whaling records for the species provide the only estimates of reproductive parameters, which have been the basis for previous studies of beaked whale energetics (e.g., Huang et al., 2011; New et al., 2013) and include: gestation (12 months) - based on fetal growth curves; lactation length (~ 1 year) - based on a single calf that had both milk and squid in its stomach; resulting in a combined estimate of calving interval (2 years), which was also supported by an accumulation of 0.5 corpora per year in mature females (Benjaminsen & Christensen, 1979).

Similar to other odontocetes, dentine GLGs in *H. ampullatus* form annually deposited layers which have been used to age individual specimens (Benjaminsen, 1972; Scheffer & Myrick, 1980). However beaked whales are unique among odontocetes in that most only have a single pair of tusk-like teeth that erupt in mature males and remain embedded in the jaw of juveniles and females (Ellis & Mead, 2017). Likely due to the difficulty in accessing tooth specimens, this study is the first investigation of ontogenetic diet shifts using stable isotopes for any species of beaked whale, based on samples from an unusually large collection (N = 151 individuals) of *H. ampullatus* teeth taken from whales that were commercially hunted in the North Atlantic.

Our primary objective was to characterize nursing duration and the end of the weaning period in individual *H. ampullatus* using $\delta^{15}N$ and $\delta^{13}C$, accounting for potential differences due to sex or regional variation. We test the hypothesis that nursing extends beyond one year, in contrast to Benjaminsen & Christensen's (1979) inference based on stomach contents of a single calf. Similar to *Physeter macrocephalus*, another deep diving cetacean with prolonged maternal care (Gero et al., 2013), beaked whales regularly dive to extreme depths (~1000m) to feed on mesopelagic and epibenthic prey (Hooker et al., 2019). As a result, juveniles may not be physically capable of independent foraging until they have grown large enough to be competent divers or engage in demanding foraging strategies, the complexities of which are currently poorly understood. Secondarily we compare differences in diet between juveniles and adults to assess whether, similar to other odontocetes (e.g. *Orcinus orca,* Newsome et al., 2009; *P. macrocephalus,* Mendes et al., 2007), there is evidence of increases in dietary trophic level with age. This study offers a rare opportunity to expand our appreciation of the variation in maternal investment strategies in beaked whales and across cetaceans.

4.4 Methods

4.4.1 Tooth Collection and Dentine Sampling

Teeth were taken from *H. ampullatus* killed by Norwegian whalers in the waters off northern Iceland in 1967 and northern Labrador in 1971 (Christensen, 1973) (Figure 4.1). Northern bottlenose whales are usually found in groups of one to four, and whalers would take all the whales they encountered, regardless of sex or age class, so we assume our dataset has low demographic capture bias (Benjaminsen & Christensen, 1979). Individuals included in this analysis ranged from 4-27 years old (median age = 14). The teeth of two *H. ampullatus* that stranded in northeast Newfoundland in 2004 were also analyzed. As specimens were part of an archived natural history collection, no approval from the University Committee on Laboratory Animals was required.

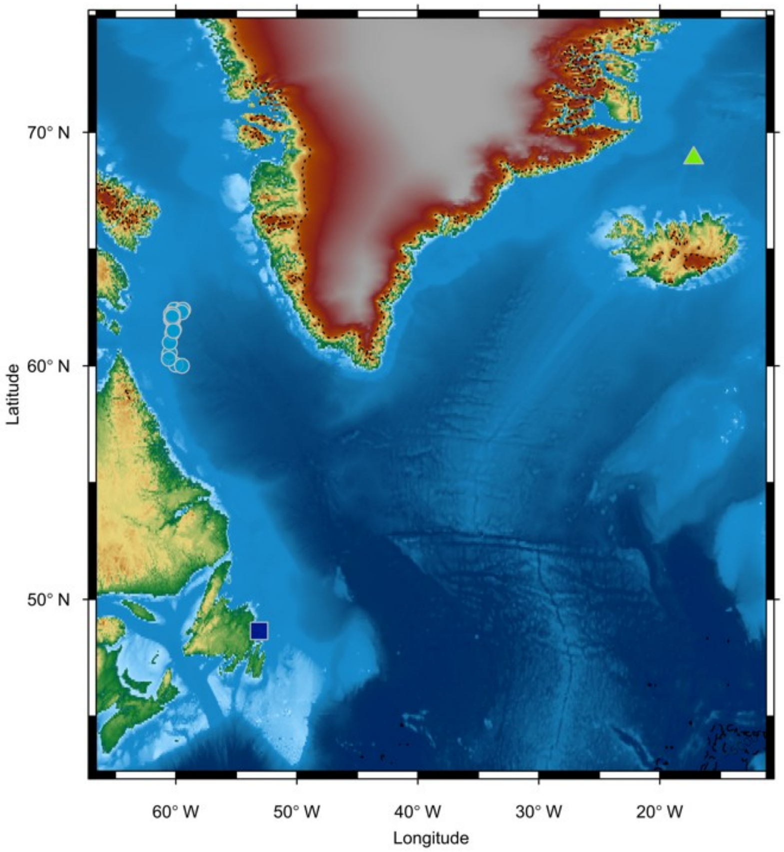


Figure 4.16. Map of study area regions and specimen collection locations. Green triangle = Iceland, light blue dots = Northern Labrador, dark blue square = Newfoundland strandings.

The jaws of whaled specimens were originally boiled for two hours to facilitate tooth extraction (Christensen, 1973). Teeth were sectioned along the longitudinal midline and stored unpreserved at room temperature in individual sachets for over 40 years prior to this study. Genetic analysis of gum-tissue from the teeth used in this study confirmed the sex documented in the whaling records (Einfeldt et al., 2019; Feyrer et al., 2019). The teeth from Newfoundland animals were extracted from decomposed specimens, air dried and stored whole until being sectioned for this study. Similar to other odontocetes (Klevezal, 1996; Luque et al., 2007; Read et al., 2018), *H. ampullatus* dentine is laminated, with one clear and one opaque layer defining each annual GLG within the cone of the tooth (Benjaminsen, 1972) (Figure 4.2). Only teeth with a clear neo-natal line and defined GLG structure across the first five years were retained for isotope analysis, reducing our sample size to 50 individuals (N = 6 from Iceland, N = 42 from Labrador, N = 2 from Newfoundland). To improve GLG definition, tooth sections were initially polished using 30µm aluminum oxide lapping film (Matthews & Ferguson, 2015) and then acid-etched using 10% formic acid (Pierce & Kajimura, 1980). GLGs were counted and aged assuming annual deposition, starting at the line that divides prenatal and postnatal dentine (Benjaminsen, 1972; Matthews & Ferguson, 2015). Using a single section of each tooth, GLGs 1-5 were sampled individually at a depth of 250-µm with a 300-µm-diameter drill bit, using a high-resolution micro-mill (New Wave Research, Freemont, California). When sufficient prenatal dentine was present it was sampled at a depth of 150 µm. For mature individuals (> 9 years old) (Christensen, 1973), we also collected samples from older GLGs as a proxy for adult diet (N = 29). However, as whales age their GLGs become compressed and are not wide enough to sample individually. Instead we collected samples representative of the mature age class by drilling across GLGs 8 -12 as a group with a 1 mm-diameter drill bit using a Dremel hand tool.

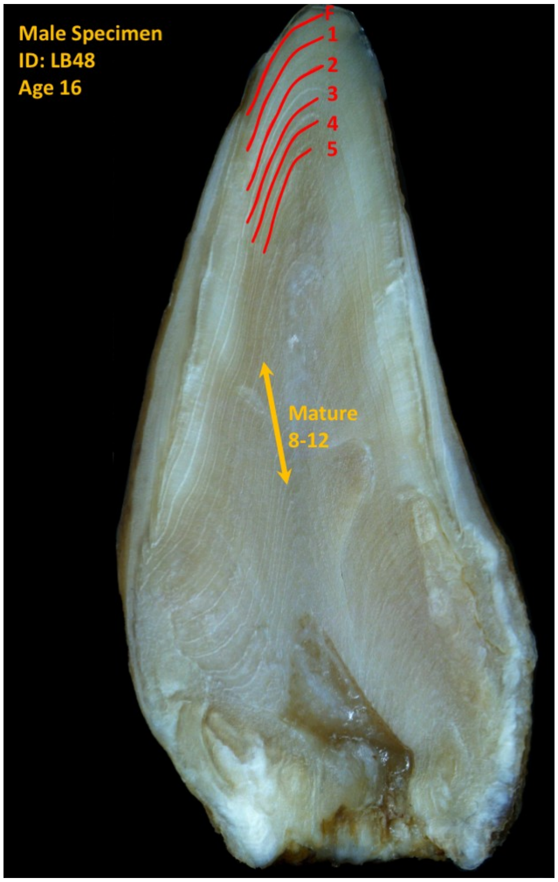


Figure 4.17. A sectioned *H. ampullatus* tooth prior to sampling. GLGs are annotated: F = fetal, 1-5 = years (red lines) and mature = sampling across years 8-12 (yellow line).

4.4.2 Stable Isotope Analysis ($\delta^{15}N$ / $\delta^{13}C$)

Powdered dentine from each sampled GLG was weighed (~1 mg) into tin cups for isotopic analysis on a Vario EL Cube elemental analyzer (Elementar, Germany) connected to a DELTA Advantage isotope ratio mass spectrometer (Thermo, Germany). Isotope ratios are reported in Delta notation (δ) as per mil (‰) deviation from isotope ratios of atmospheric N_2 for nitrogen and Vienna Pee-Dee Belemnite (V-PDB) limestone for carbon. $\delta^{15}N$ or $\delta^{13}C$ are defined as $\delta = (R_{sample} - R_{standard})/R_{standard})$, where R is the ratio of the abundance of the heavy to the light isotope. Values are normalized to internal standards nicotinamide, ammonium sulfate + sucrose, caffeine, and glutamic acid, whose isotopic compositions cover the natural range of samples ($\delta^{15}N$ -16.61 to 16.58‰, $\delta^{13}C$ -34.46 to –11.94‰) and are calibrated to international standards IAEA-N1(+0.4‰), IAEA-N2(+20.3‰), USGS-40(-4.52‰) and USGS-41(47.57‰) for $\delta^{15}N$, and IAEA-CH-6(-10.4‰), NBS-22(-29.91‰), USGS-40(-26.24‰) and USGS-41(37.76‰) for $\delta^{13}C$. Analytical precision based on repeated measures of laboratory reference materials not used in calibrations was ~0.1‰ for both $\delta^{15}N$ and $\delta^{13}C$ within multiple laboratory runs. Variation between duplicate measures of ~10% of samples had an absolute mean of 0.26 ‰ for $\delta^{15}N$ and 0.21 ‰ for $\delta^{13}C$.

The small size of some GLGs meant it was sometimes necessary to collect amounts less than 1 mg. A linearity study showed samples <0.5 mg appeared to have a positive bias in $\delta^{15}N$ but not $\delta^{13}C$, and further analysis was restricted to samples weighing >0.5mg, reducing the number of GLG samples available for some individuals. Additionally, we omitted the smallest duplicate sample, so that only a single sample from an individual GLG was included in further analysis (Supplementary data; Feyrer et al., 2020).

4.4.3 Data Analysis

Following the screening for duplicates and sample weight described above, 50 individuals were included in summary statistics regardless of how many GLGs were available. However, ontogenetic trend analysis was restricted to those individuals which had stable isotope data available from at least GLGs 1-3 (N = 37). Data structure, variables, and sample sizes are identified in Table 4.1 and variable inclusion rationale and data sources are further described in Appendix C (Table C1).

Table 4.13. Data structure, variables and sample sizes

Dependent variables	**Independent variables**	**Total individuals N = GLG samples**	**GLG chronologies N = GLG samples**
$\delta^{15}N$ $\delta^{13}C$	Region GLG – Year, Age Class Sex Individual Age	50 IDs N = 244 GLGs (*288 including duplicate samples)	39 IDs N = 207 GLGs

For comparison with other published values and ecological studies, carbon isotope values were adjusted for the oceanic Suess effect, applying a factor of 0.0019‰ yr^{-1} to $\delta^{13}C$ measured in GLGs; $\delta^{13}C_{cor}$ values are approximately relative to the year 2000

(English et al., 2018; Matthews & Ferguson, 2015; Quay et al., 2003). The isotope values sampled from a cross section of mature GLGs (age 8-12) were assumed to represent the average isotopic profile of adult whales, and used as a benchmark for assessing when the weaning associated $\delta^{15}N$ decline ended.

The dataset was initially summarized and explored for the presence of ontogenetic trends in nitrogen and carbon isotope ratios. The effect of sex and region on isotopic composition was initially evaluated using two-sample t-tests. A hierarchical linear mixed effects regression model implemented with the lme4 package in R (Version 3.0.1, Bates et al., 2015) assessed the effects of sex, region and GLG. Given uneven sample sizes between GLGs, we used a paired t-test to consider the distinction between subsequent GLGs. Due to the small sample size (N = 2) and differences in source collection from other samples, Newfoundland specimens were not included in statistical summaries or tests unless specified.

To investigate ontogenetic trends and nursing duration, for each individual with samples from GLGs 1-3 (N = 37) we calculated the ‰ difference between GLG 1 and all other available GLGs (fetal dentine, GLGs 2-maturity). Three methods of determining weaning completion were compared for individuals which had samples collected from mature age classes by calculating the age: (A) when $\delta^{15}N$ values stopped decreasing (e.g. the lowest value of $\delta^{15}N$ in the chronology, Newsome et al., 2010); (B) when $\delta^{15}N$ was equal to the value for their mature age class value (+/- 0.25 ‰) (Newsome et al., 2009); and (C) when $\delta^{15}N$ was -1.2‰ lower than GLG1(+- 0.25 ‰) (Matthews & Ferguson, 2015; Newsome et al., 2009). The threshold for (C) was based on an average ‰ difference between GLG1 and mature samples in this study, and similar differences found in other studies of weaning in odontocetes (Matthews & Ferguson, 2015; Newsome et al., 2009). For each method, individual age at weaning completion was compared by sex and between Labrador and Iceland regions using a two-sample t-test. Small sample size for Newfoundland precluded inclusion in significance tests.

4.5 Results

4.5.1 Nitrogen

Across individual chronologies, we found $\delta^{15}N$ generally peaked in GLG1 (mean = 17.73, SE = 0.10) and then declined with age. Within individuals, the relative decline in $\delta^{15}N$ between GLG 1 and all other GLG years averaged – 1.02 ‰ (Figure 4.3 a). GLG 1 $\delta^{15}N$ was higher (mean = 0.93 ‰) than fetal dentine (mean = 17.00, SE = 0.16) and 1.06 ‰ higher than mature age class values (mean = 16.62, SE = 0.09). $\delta^{15}N$ values across all GLGs from Labrador and Iceland ranged ~3.8 ‰ (15.16 to 19.0‰). For the two specimens from Newfoundland, $\delta^{15}N$ spanned 4.8‰ and was lower (range 12.9 – 17.7‰) than average values from Labrador and Iceland. For GLGs > 1, both Newfoundland specimens were greater than 1 standard deviation lower in $\delta^{15}N$ than other regions, with the adult female ~3‰ lower across GLGs.

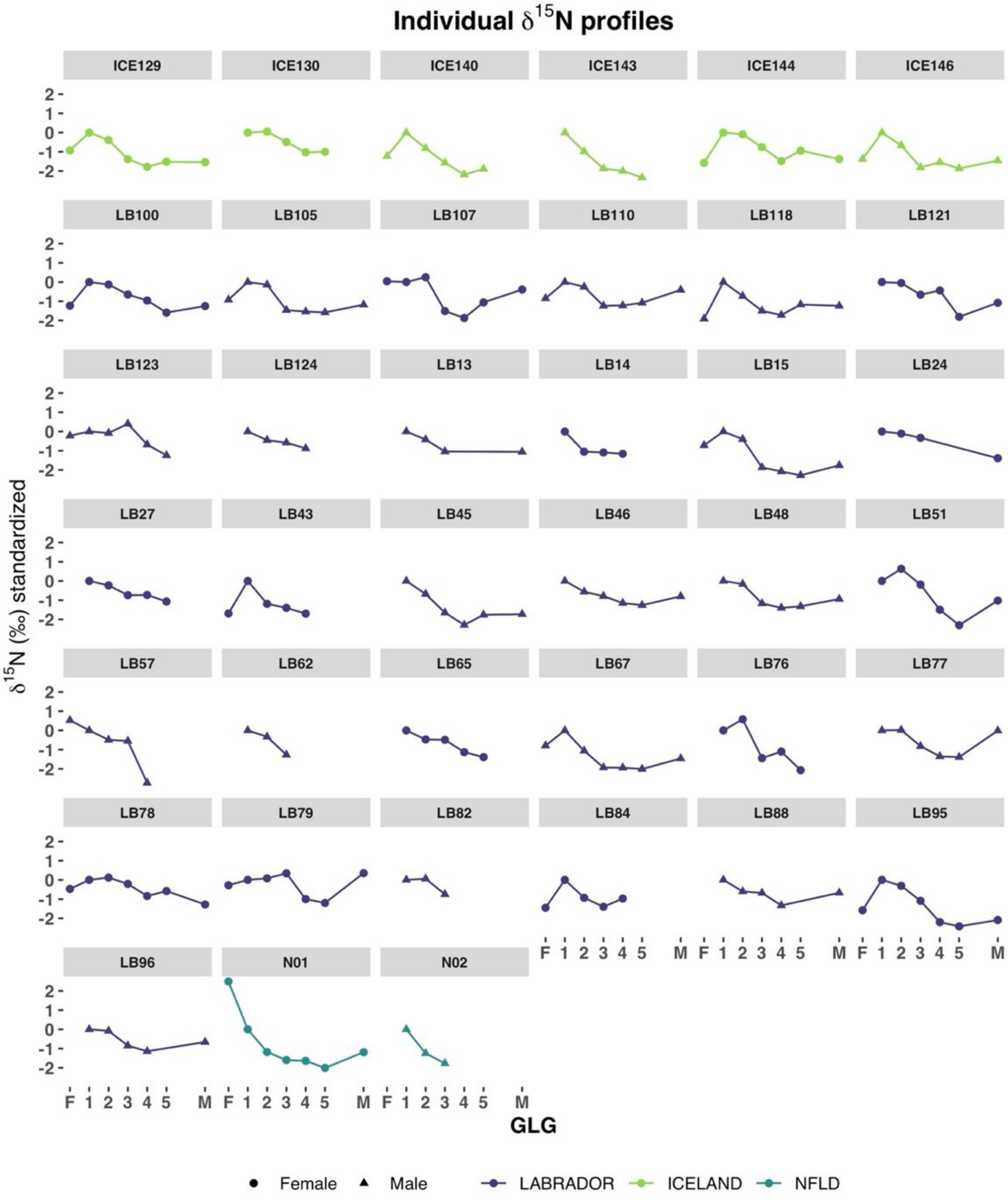
4.5.2 Carbon

$\delta^{13}C_{cor}$ values generally increased with age (mean increase in $\delta^{13}C_{cor}$ per GLG = 0.37, fetal to maturity). GLG1 was on average more enriched in $^{13}C_{cor}$ (+0.51 ‰, mean = -14.07) than fetal dentine (mean = -14.48), and more depleted than older GLGs. The range of $\delta^{13}C_{cor}$ values for mature samples were on average 1.06 ‰ higher than GLG 1 (Figure 4.3 b). Between regions, $\delta^{13}C_{cor}$ in Labrador and Iceland were higher (-15.66 to -12.57 ‰) than Newfoundland (-17.17 to - 14.78‰). The juvenile male whale from Newfoundland was one notable exception to the overall ontogenetic increase in carbon, as his $\delta^{13}C_{cor}$ values declined from GLG 1 to 3 (Figure 4.3 b). Across all GLGs, $\delta^{13}C_{cor}$ values for the Newfoundland specimens were > 1 standard deviation below Labrador or Iceland specimen GLGs.

4.5.3 Influence of Sex, Region and GLG

Average values of $\delta^{15}N$ and $\delta^{13}C_{cor}$ for females and males had considerable overlap and did not demonstrate a consistent pattern or significant difference between sexes across GLG's (Figures 4.4 a and b, $t = 0.85$, df = 53.2, $p = 0.39$). Differences in values of $\delta^{15}N$ and $\delta^{13}C_{cor}$ between Labrador and Iceland were not significant (Figures 4.5 a and b, $t = 0.58$, df = 12.1, $p = 0.57$).

(a)


Individual δ15N profiles
ICE129
ICE130
ICE140
ICE143
ICE144
ICE146
LB100
LB105
LB107
LB110
LB118
LB121
LB123
LB124
LB13
LB14
LB15
LB24
LB27
LB43
LB45
LB46
LB48
LB51
LB57
LB62
LB65
LB67
LB76
LB77
LB78
LB79
LB82
LB84
LB88
LB95
LB96
N01
N02
δ15N (‰) standardized
2
1
0
-1
-2
F 1 2 3 4 5 M
GLG
Female
Male
LABRADOR
ICELAND
NFLD

(b)

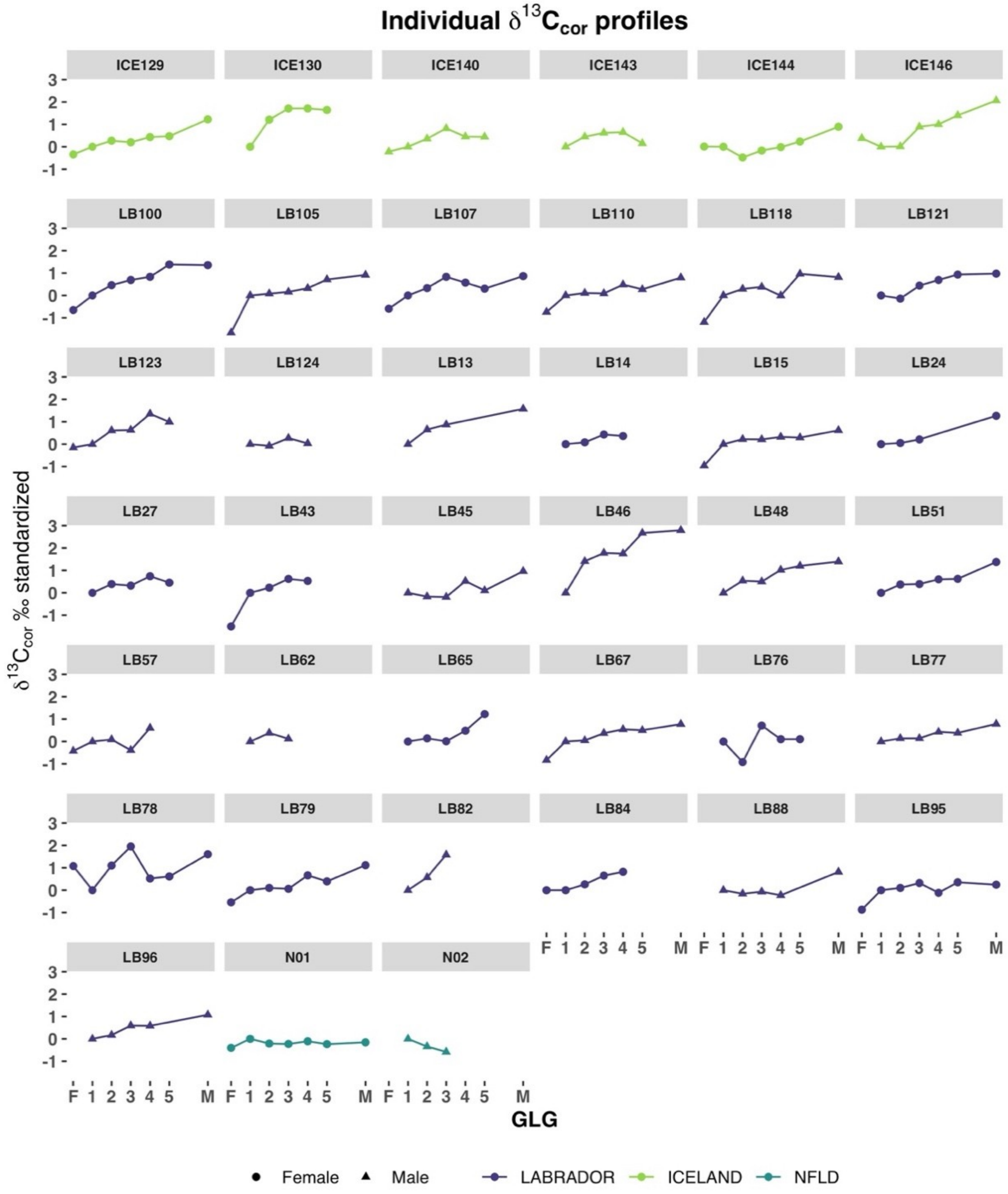
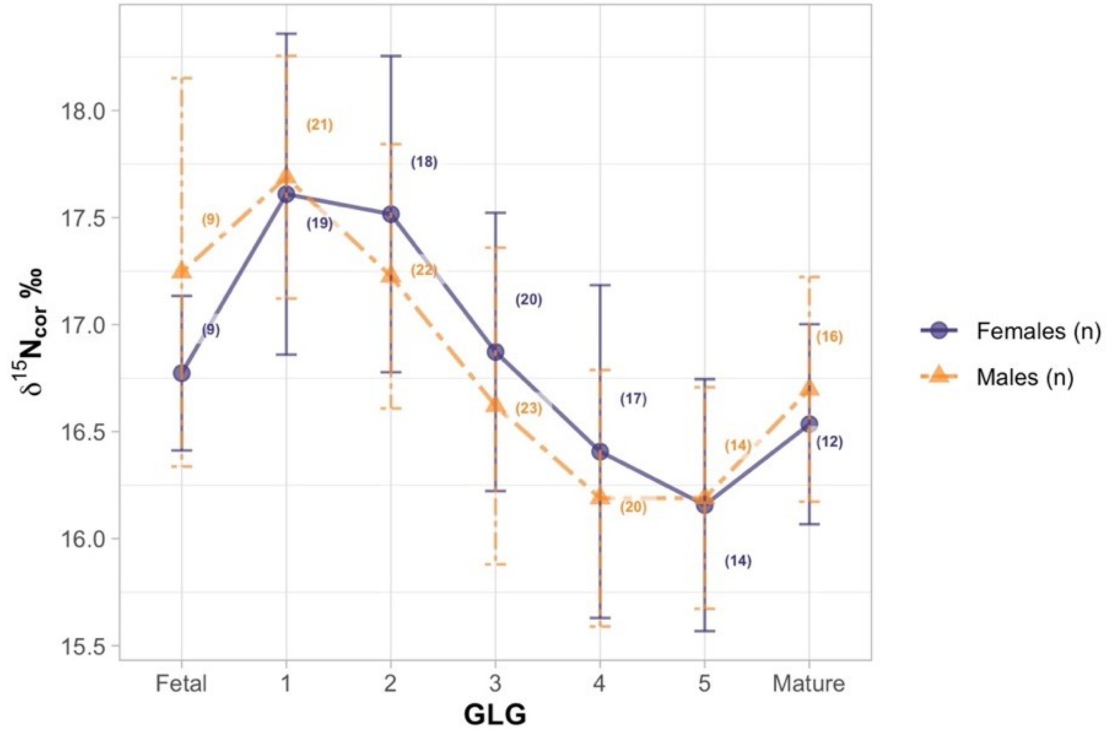


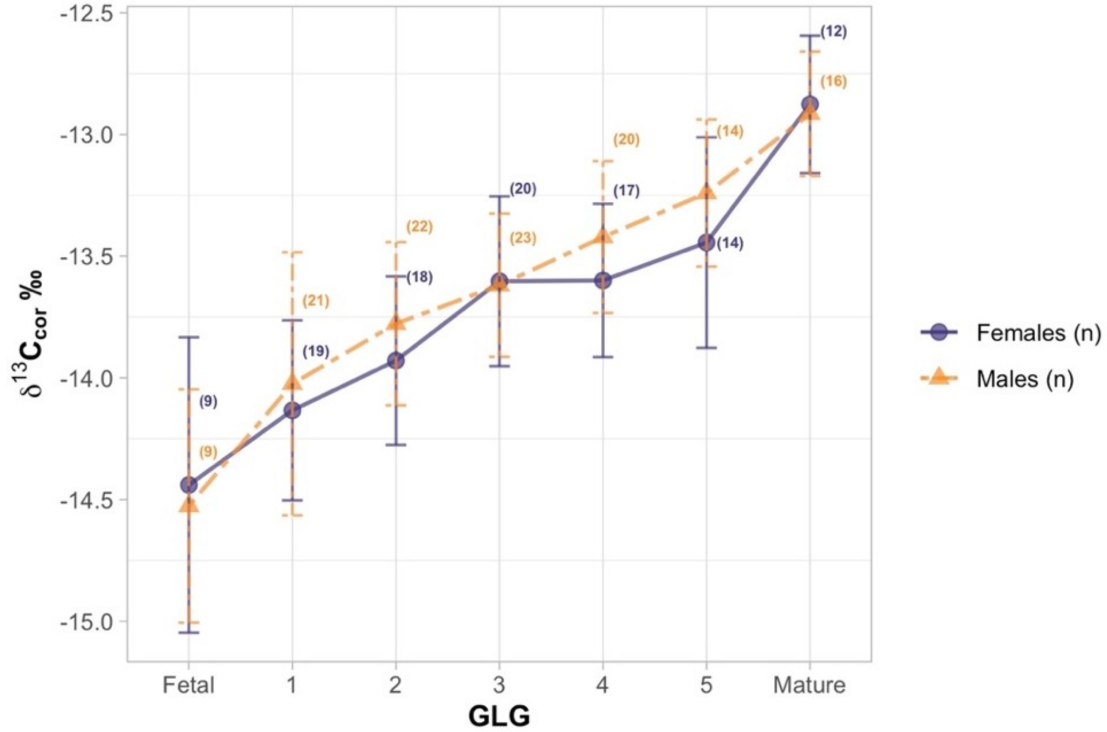
Figure 4.18 Individual chronologies for (a) δ ^{15}N and (b) δ ^{13}C for each region. Regions are indicated by colour. Isotope values were standardized to be relative to GLG 1 for prior (fetal dentine = F) or subsequent (years 2 - mature = M) GLGs. Sex of specimen is indicated by circle (female) and triangles (male).

(a)



δ15Ncor ‰
18.0
17.5
17.0
16.5
16.0
15.5
Fetal
1
2
3
4
5
Mature
GLG
(9)
(9)
(21)
(19)
(18)
(22)
(20)
(23)
(17)
(20)
(14)
(14)
(16)
(12)
Females (n)
Males (n)

(b)



δ13Ccor ‰
-12.5
-13.0
-13.5
-14.0
-14.5
-15.0
Fetal
1
2
3
4
5
Mature
GLG
(9)
(9)
(21)
(19)
(22)
(18)
(20)
(23)
(20)
(17)
(14)
(14)
(12)
(16)
Females (n)
Males (n)

Figure 4.19 Ontogenetic trends in average (a) δ^{15}N and (b) δ^{13}C by sex. Females (N = 109 GLG samples) are purple points and males (N = 125 GLG samples) are orange triangles. Whisker bars represent standard deviation. Iceland and Labrador samples only.

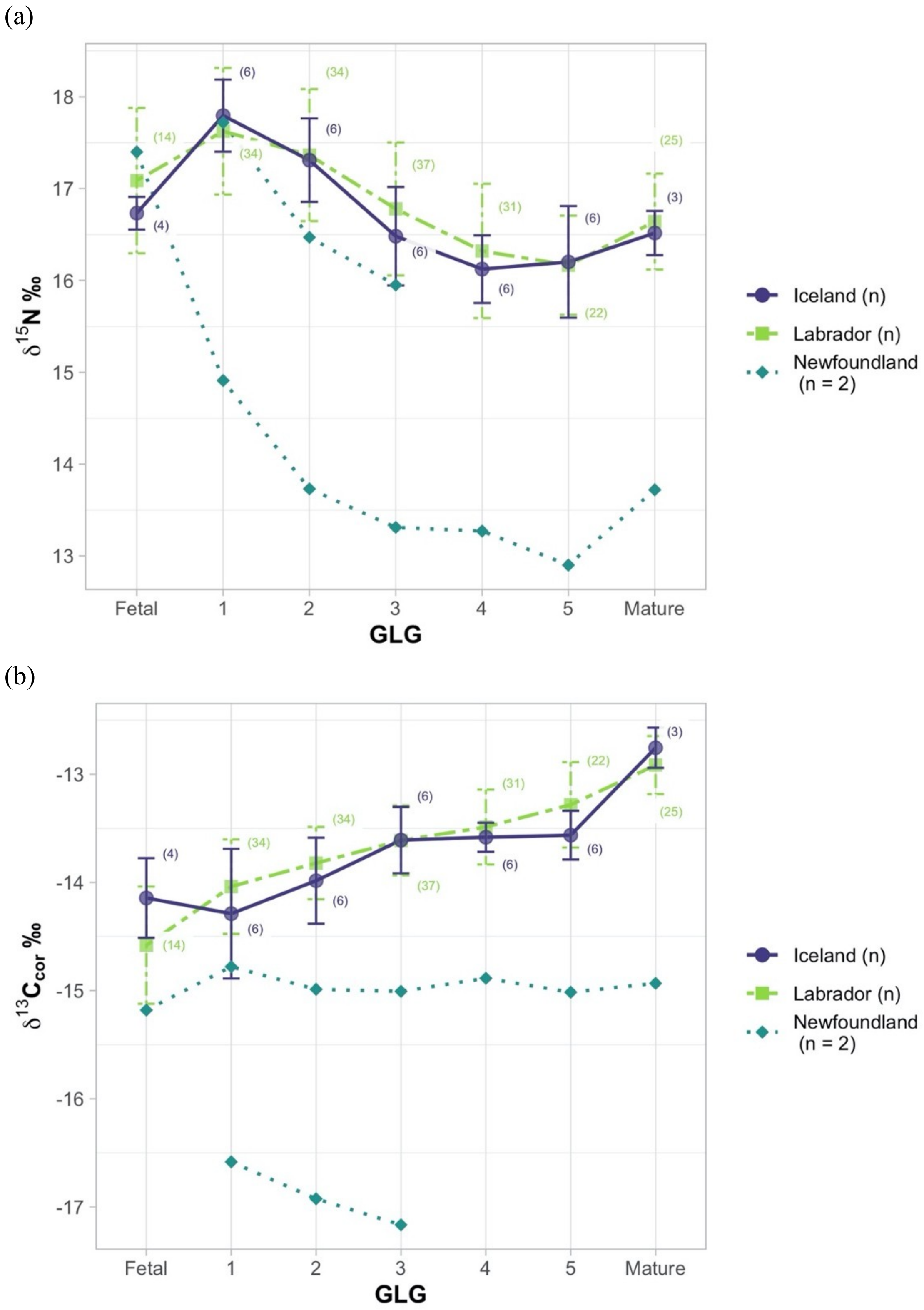

(a)
18
17
16
15
14
13
δ^{15}N ‰
Fetal
1
2
3
4
5
Mature
GLG
(14)
(4)
(6)
(34)
(34)
(6)
(37)
(6)
(31)
(6)
(6)
(22)
(25)
(3)
Iceland (n)
Labrador (n)
Newfoundland (n = 2)
(b)
-13
-14
-15
-16
-17
δ^{13}C$_{cor}$ ‰
Fetal
1
2
3
4
5
Mature
GLG
(4)
(14)
(34)
(6)
(34)
(6)
(6)
(37)
(31)
(6)
(22)
(6)
(3)
(25)
Iceland (n)
Labrador (n)
Newfoundland (n = 2)

Figure 4.20 Ontogenetic trends by region for values of (a) $\delta^{15}N$ and (b) $\delta^{13}C$. Purple points and green squares are mean values with standard deviation for Labrador and Iceland, blue diamonds are individual values of two specimens from Newfoundland.

Mixed effects models, implementing individual as a random effect, compared eight different combinations of fixed effects including GLG, Region and Sex (Tables 4.2 a and b). Only Region and GLG were retained in the best fit mixed effect models for predicting relative $\delta^{15}N$ and $\delta^{13}C$ values. Model fit, assessed using Δ AIC ≦ 2, indicated GLG was important for explaining both $\delta^{15}N$ and $\delta^{13}C$, Region was included in all best fit models for $\delta^{15}N$ and in one model for $\delta^{13}C$, Sex was also included in one of the best models for $\delta^{13}C$ (Table 4.2). Given the overlap in mean values with standard error between Labrador and Iceland and between males and females we conclude that the influence of region and sex on isotopic profiles are small relative to the variation attributed to GLG (age) and individual.

Table 4.14. Mixed effect model results comparisons for (a) $\delta^{15}N$ and (b) $\delta^{13}C$. Best fit models are indicated in bold based on lowest AIC score and Δ AIC ≦ 2. BIC and Log Likelihood (logLik) scores with degrees of freedom (df) are included for comparison. " (1|ID)" indicates an individual effect.

(a) $\delta^{15}N$	**ΔAIC**	**AIC**	**BIC**	**logLik**	**df**
~GLG_N+Region +(1|ID)	0.0	329.3	345.1	-159.6	5
~Sex +GLG_N+Region +(1|ID)	0.5	329.8	348.7	-158.9	6
~GLG_N*Region +(1|ID)	1.5	331.3	350.2	-159.6	6
~GLG_N +(1|ID)	2.3	331.6	344.2	-161.8	4
~Sex*GLG_N+Region +(1|ID)	2.4	331.6	353.8	-158.8	7
~Sex*GLG_N+(1|ID)	2.7	334.3	353.3	-161.1	6
~Sex+GLG_N +(1|ID)	3.2	332.5	348.3	-161.2	5
~Sex+Region +(1|ID)	25.1	354.3	370.1	-172.2	5
~1 +(1|ID)	26.9	356.2	365.7	-175.1	3
(b) $\delta^{13}C$	**ΔAIC**	**AIC**	**BIC**	**logLik**	**df**
~GLG_N +(1|ID)	0.0	198.9	211.6	-95.5	4
~GLG_N+Region +(1|ID)	1.0	199.9	215.7	-94.9	5
~Sex+GLG_N +(1|ID)	2.0	200.9	216.7	-95.4	5
~GLG_N*Region +(1|ID)	2.2	201.1	220.0	-94.5	6
~Sex*GLG_N +(1|ID)	2.7	201.6	220.6	-94.8	6
~Sex +GLG_N +Region+(1|ID)	2.9	201.8	220.8	-94.9	6
~Sex*GLG_N +Region+(1|ID)	3.6	202.5	224.6	-94.3	7
~1 +(1|ID)	135.8	334.7	344.2	-164.3	3

~Sex + Region + (1|ID)	139.0	337.9	353.7	-164.0	5

Paired t-tests assessing the difference between $\delta^{15}N$ and $\delta^{13}C$ of an individual between consecutive GLG's found significant differences between $\delta^{15}N$ in GLG pairs 1 through 4 and between $\delta^{13}C$ in GLG pairs Fetal (F) through age 3 (Table 4.3). GLGs 5 and mature (M) were also significantly different for both isotopes.

Table 4.15. Paired t-test results for comparisons between GLG years within individuals for (a) $\delta^{15}N$ and (b) $\delta^{13}C$. Test significance (p-value), mean difference in ‰ (Mean dif. ‰), confidence intervals of the difference (C.I. ‰) and degrees of freedom (df) are presented for each test.

(a) $\delta^{15}N$

GLG	p-value	Mean dif. (‰)	C.I. (‰)		df
F to 1	0.005	-0.72	-1.23	-0.26	18
1 to 2	<0.001	0.37	0.22	0.52	38
2 to 3	<0.001	0.65	0.50	0.81	40
3 to 4	<0.001	0.40	0.24	0.57	37
4 to 5	0.322	0.09	-0.10	0.28	28
5 to M	0.001	-0.49	-0.74	-0.23	20

(b) $\delta^{13}C$

GLG	p-value	Mean dif. (‰)	CI (‰)		df
F to 1	0.003	-0.51	-0.81	-0.20	18
1 to 2	0.002	-0.22	-0.36	-0.09	38
2 to 3	<0.001	-0.24	-0.36	-0.12	40
3 to 4	0.225	-0.09	-0.23	0.06	37
4 to 5	0.086	-0.13	-0.28	0.02	28
5 to M	<0.001	-0.35	-0.53	-0.17	20

4.5.4 Weaning completion

Nursing duration ranged across methods with median age of three to five. Method (A) provided older estimates of weaning completion (mean = 4.5), while methods (B) and (C) suggested weaning was completed earlier, with mean ages of 3.4. There was no substantial difference in nursing duration between Labrador or Iceland regions or with sex (Table 4.4) using any of the weaning analysis methods.

Table 4.16. Mean, median and range of weaning completion age for different estimation methods as described in the analyses, compared by (a) sex and (b) region.

(a) Estimation Method	Sex	Mean GLG	Median GLG	GLG Range (yrs)
A	F	4.6	5	4 - 5
	M	4.4	5	3 - 5
B	F	3.7	4	2 - 5
	M	3.1	3	2 - 5
C	F	3.4	3	2 - 5
	M	3.1	3	2 - 5
(b) Estimation Method	**Region**	**Mean GLG**	**Median GLG**	**GLG Range (yrs)**
A	Iceland	4.3	4	4 - 5
	Labrador	4.5	5	3 - 5
B	Iceland	4.0	4	3 - 5
	Labrador	3.1	3	2 - 5
C	Iceland	2.7	3	2 - 3
	Labrador	3.5	3	2 - 5

4.6 Discussion

We conclude that *H. ampullatus* have a prolonged nursing period, based on a slow decrease in $\delta^{15}N$ over GLGs 1-5. This decline was generally consistent across regions (N = 50 individuals) and between sexes (N = 48 individuals) and based on a chronological analysis of 39 individuals we found that weaning ends on average between ages three and four. Extended maternal care has not previously been documented in a beaked whale species and is in contrast to the only other estimate for *H. ampullatus* completing nursing in their first year, which was based on the stomach contents for a single calf (Benjaminsen, 1972). This new evidence of extended care in *H. ampullatus* has implications for the life history and energetics of other species of beaked whales, as well as their ability to recover from the effects of whaling or other population level impacts such as disease or mass stranding events due to mid-frequency active sonar (MFAS) (Simonis et al., 2020).

While the nursing duration varies widely across mammal taxa, it is known to be generally related to maternal body size, as prolonged nursing helps fulfill the caloric requirements for growth of larger independent animals (Hayssen & Orr, 2017; Oftedal, 1997). Weaning typically occurs when offspring reach a certain size, and while beaked whales have proportionally larger calves compared to other cetaceans (Huang et al., 2011), between birth and age five juvenile *H. ampullatus* almost double their length from three to six meters, with adult whales reaching 7-9 meters (Benjaminsen & Christensen, 1979). Although the calves of the largest odontocete, *P. macrocephalus*, are relatively smaller at birth, (~ 33% of maternal size), they have prolonged lactation and nursing (mean 36 months, range 2-13 years (Best et al., 1984; Gero et al., 2013), presumably to support their growth and development. Due to the large calf size of beaked whales and prior assumptions of their short nursing duration and inter-calf intervals, it has been suggested that their reproduction somewhat resembles the capital breeding energetics of baleen whales (e.g., Huang et al., 2011; New et al., 2013). Unlike beaked whales, however, baleen whales are bulk feeders able to ingest large amounts of food over short time periods (Goldbogen et al., 2017), limited by life history attributes tied to the seasonal constraints of migration and ocean productivity, and have significantly higher average milk fat percent to support the rapid growth, development and weaning of their calves (Hayssen & Orr, 2017; Oftedal, 1997).

Although the composition of whale milk is poorly documented across species, odontocetes are generally known to have energetically less rich milk (mean fat = 24%) than baleen whales (mean fat = 33%) (Hayssen & Orr, 2017; Oftedal, 1997). The only two records available for beaked whales suggest their milk fat % is even lower than average for odontocetes, based on single records of specimens of *H. ampullatus* (20%) and *Mesoplodon stejnegeri* (17%) (Oftedal, 1997). However, milk energy output is not strictly based on fat composition, as solids (protein, sugars and ash or minerals) also contribute to total calories available for consumption. For the odontocetes where total milk energy output has been calculated (*P. macrocephalus*, *Kogia breviceps, Delphinus delphis*, Oftedal, 1997), it is notably low, comparable only to values found in primates, which are also known to have long lactations and extended periods of dependency.

While data are not available to calculate the energetic output of *H. ampullatus* milk, similar to other medium to large odontocetes, we suggest that prolonged nursing contributes to the caloric demands of rapid juvenile growth in the first 3 to 5 years.

Beyond providing necessary nutrition, nursing in mammals serves multiple functions; cetacean calves depend on nursing for their thermoregulation in the conversion of high fat milk into blubber, and maternal proximity offers protection from predators, ongoing socialization, and other important learning opportunities such as foraging and migration routes (Hayssen & Orr, 2017). Prolonged nursing and gradual weaning, as part of the transition to nutritional independence, could be a life history adaptation for odontocetes with complex foraging strategies, such as deep diving. Both the biological demands and technical skills of foraging at depth may require time for physiological development and social learning. Although Newsome et al.'s study (2010) of *P. macrocephalus* GLGs, found a gradual decrease in δ^{15}N over the first 5 years, indicative of prolonged nursing, depth-recording tags indicated 1-year old calves had the capacity to dive to depths and durations of adult whales (Tønnesen et al., 2018). Whether *H. ampullatus* calves are also capable of diving to depths recorded for adult whales (e.g. 800 -1400m, Hooker & Baird, 1999) is currently unknown. However, as juvenile beaked whales are overrepresented in mass stranding events linked to naval sonar, Hooker et al. (2009) suggested that other aspects of dive capacity such as body mass, lung volume, or endurance for repeated dives, may be developmentally limited. We do know that for many species with a single precocial offspring, their young are introduced to solid food early despite prolonged nursing (Hayssen & Orr, 2017). Thus, the need for prolonged maternal care in deep divers may also relate to the technical, socially learned aspects of foraging at depth, such as prey identification, capture and coordination with conspecifics.

While most isotopic studies of ontogeny have focussed on differences in ^{15}N, here we also observed a regular pattern of increasing δ^{13}C values from GLG 1 to older GLGs, which we suggest is consistent with weaning physiology. Milk is rich in ^{13}C-depleted lipids, which if they are being incorporated into proteins, would lead to nursing animals having lower δ^{13}C values than adults (Borrell et al., 2015; DeNiro & Epstein, 1977; Newsome et al., 2006). Although the trend for carbon is consistent with our inferences of prolonged nursing and a gradual transition from milk to solid food, gradual enrichment in ^{13}C has not always been observed in other studies of odontocetes (e.g. *D. leucas*, Matthews & Ferguson, 2015; *Grampus griseus*, Evacitas et al., 2017). As juvenile *H. ampullatus* whales learn to forage deeper, the increase in $\delta^{13}C$ may reflect increasing consumption of bentho-pelagic species, which would be expected to have higher $\delta^{13}C$ values (Trueman et al., 2014). Baseline $\delta^{13}C$ can also vary spatially with latitude (Trueman & St John Glew, 2018), and if all individuals demonstrated an ontogenetic shift in distribution it could potentially cause an increase or decrease in $\delta^{13}C$ observed in tissues (e.g. (Trueman et al., 2019). However, based on global ^{13}C isoscapes models (Magozzi et al., 2017), the lower latitudes (< 40°) where substantial foraging would have to occur to influence their $\delta^{13}C$ profile, are at least 20°south of northern Labrador and Iceland, and outside of the known southern limit for the range of this species.

The patterns we observed appear largely consistent across a large number of specimens, however as a result of only including teeth with clearly defined GLG structure, we accept that our estimate of nursing duration may be biased towards healthy individuals. It is possible that age at weaning completion could be underestimated if the individuals in the study were weaned earlier due to available resources, or overestimated if maternal investment was longer than average. As our primary dataset included animals of different ages with a range of birth years spanning 1944 - 1967 (i.e. over four decades) it is unlikely either of these factors biased our results. The distinct GLG $\delta^{15}N$ and $\delta^{13}C$ patterns in the two whales which stranded in Newfoundland suggest that both individuals weaned earlier than the other specimens (at age 1-2, Figure 4.3 a). Although there is no clear understanding of the relationship between $\delta^{13}C$ and poor health conditions such as disease in whales, blubber stores may be mobilized during starvation or fasting (e.g *Ursus americanus*, Ahlquist et al., 1984), and ^{13}C depleted lipids would be incorporated in incremental tissues such as dentine. A notably decreasing rather than increasing $\delta^{13}C$ trend (Figure 4.3 b) in the stranded male whale could reflect a longer period of physiological decline. Absolute isotopic values of carbon and nitrogen also suggest that the diet of Newfoundland whales was distinct from the specimens killed in Labrador and Iceland 30 years prior (Figures 4.5 a and b). While we attempted to account for known climatic trends in $\delta^{13}C$ (i.e. Suess effect) by adjusting our $\delta^{13}C$ values, other temporal influences we cannot account for, such as other baseline isotope or other ecosystem shifts, may have occurred across the North Atlantic during the ~ 30-year period separating specimens. This highlights some of the challenges in using stranded animals of unknown health status and specimens from disparate time periods to make broad inferences on poorly understood species biology. Further investigation on the relationship between health status and the appearance of GLG structure in marine mammals would help clarify the influence of these factors for future studies.

Interestingly, our finding that $\delta^{15}N$ in fetal dentine was almost 1 ‰ lower than GLG 1 differs from the pattern of steady decline in $\delta^{15}N$ from a peak in fetal dentine observed in other species of cetaceans (*Grampus griseus*, Evacitas et al., 2017; *Monodon monoceros*, Zhao et al., unpublished data; *D. leucas*, Matthews & Zhao, unpublished data). Our explanations for the inconsistencies between enrichment patterns in fetal dentine across cetacean species consider two possibilities: (1) if tissues measured in other studies are actually neonatal rather than fetal dentine, $\delta^{15}N$ for other species would reflect an ongoing decline in post-partum nursing (Riofrío-Lazo et al., 2012); or (2) differences are due to species-specific reproductive biology, such as physiological differences between capital and income breeders or growth dependent trophic enrichment factors. While occasional errors in identification of fetal dentine may occur, as Stewart & Stewart (2010) describe there are multiple established landmarks for distinguishing pre and post-natal dentine deposition, making it unlikely that this is the source of consistent error across studies. Borrell et al. (2015) found fetal tissues of capital breeders, which sustain reproduction with stored fat reserves, were higher in $\delta^{15}N$ than their mothers, whereas for income breeders, mother-fetus $\delta^{15}N$ discrimination was not observed. While odontocetes are generally recognized as income breeders, as per Huang et al. (2011), aspects of *H. ampullatus* prenatal reproductive energetics, such as large relative calf size, do not align with the other odontocete species. Alternatively, if growth dependent ^{15}N enrichment occurs due to rapid development in utero, it could explain fetal $\delta^{15}N$ patterns, which may be different in smaller cetacean species than for larger species such as *H. ampullatus*. The inconsistencies in fetal development between species highlight the need to better understand the influence of maternal physiology on fetal development and stable isotope discrimination so that future studies can accurately interpret stable isotope profiles (Borrell et al., 2015).

Theory predicts that parents in polygynous species may adopt a sex-bias in infant investment towards males (Maynard Smith, 1980). While we do not have a good understanding of the mating systems across any of the species of beaked whales (Baird, 2019), most are sexually dimorphic, and in *H. ampullatus*, males are significantly larger in size, suggesting they need additional energetic resources for growth (Clutton-Brock et al., 1985). Although Hooker et al. (2001) found adult males were marginally enriched in ^{15}N relative to females, we did not find significant evidence that this occurs as part of maternal investment. While there may be some influence of sex on trophic position in mature animals, there was no difference between males and females in terms of nursing duration, or relative values of $\delta^{15}N$ or $\delta^{13}C$ across GLGs. However, individual variation and annual averaging within GLGs may mask the presence of finer scale sex-based patterns or trends in isotopic enrichment (Figures 4.3 a and 4.4) (Smith et al., 2020).

The weaning period, which includes the introduction to solid food accompanied by nursing, can vary in length depending on whether maternal weaning strategies are abrupt or gradual. Using the timing associated with the cessation of a general declining trend in $\delta^{15}N$, changepoint analysis or model fit against a number of theoretical curves, a number of authors (Evacitas et al., 2017; Matthews & Ferguson, 2015; Newsome et al., 2009) have proposed that unlike baleen whales, weaning in odontocetes is a relatively gradual process. While the introduction of solid food may occur within the first year, this is often accompanied by prolonged nursing across a number of odontocete species (Whitehead & Mann, 2000), suggesting stomach contents are unlikely to provide good evidence of the age when weaning is complete. The point when $\delta^{15}N$ values become relatively stable and more consistent with subsequent GLGs or are approximately equivalent to mature baseline values has been used to estimate weaning completion (Matthews & Ferguson, 2015; Newsome et al., 2009; Riofrío-Lazo et al., 2012). For *H. ampullatus*, we found generally similar results across methods, suggesting weaning was complete when whales were between 3 to 4 years old. The point when $\delta^{15}N$ values stopped decreasing (Method

A), suggested $\delta^{15}N$ declined into year five for some individuals, which could reflect individual variation in prolonged nursing, or differences in ability to forage on higher trophic level prey. Defining weaning completion as the point when $\delta^{15}N$ was equal to mature values (Method B), or when $\delta^{15}N$ was 1.2‰ lower than GLG1 (Method C), suggests that in *H. ampullatus*, similar to other odontocetes, nursing is prolonged with weaning taking over 3 years to complete.

If Benjaminsen (1972) was correct in their calculation of a 12 month gestation period for *H. ampullatus*, nursing a calf for at least three years would double previous estimates of their reproductive cycle to at least four years (Benjaminsen & Christensen, 1979). New et al.'s (2013) bioenergetic models of beaked whales found that low survival and reproduction was tied to the relatively short estimates for duration of lactation, and the assumption of a 2-year calving interval. Energetically, a large percentage of beaked whales in New et al.'s (2013) models had difficulty meeting their metabolic requirements under standard assumptions and inferred reproductive parameters derived from historical whaling data. Prolonged nursing was identified by New et al. (2013) as an alternate strategy that would give females a recovery period between mating, allowing them to rebuild energetic stores and increase the probability of their next calf's survival. Prolonged maternal investment and a longer inter-calving interval also has consequences for the rate of effective population growth. Given the assumption that for most odontocetes, pregnancy and lactation rarely overlap, extended nursing decreases the lifetime reproductive potential of the species by half. For *H. ampullatus*, extended maternal care would prolong their recovery from commercial whaling and increase the impact of contemporary risks to their populations such as disease outbreaks, MFAS induced strandings or other unusual mortality events (Barley et al., 2007; Grove et al., 2020; Hayssen & Orr, 2017; New et al., 2013; Simonis et al., 2020; Whitehead & Mann, 2000).

A longer nursing period also implies that *H. ampullatus* have extended maternal associations, and suggests that social structure of beaked whales may be more complex than previous observational studies have been able to detect (Baird, 2019; Gowans et al., 2001). Generally beaked whales are found in very small groups and are not considered particularly social, however in well studied beaked whales (e.g. *Ziphius cavirostris*, *Berardius* spp., *Mesoplodon densirostris*), there is some evidence of long-term bonds (over months to years) between individuals using photo-ID methods (Baird, 2019; Hooker et al., 2019). Although McSweeney (2007) documented repeated associations over two years between a female *Ziphius cavirostris* and her calf, and Baird (2019) suggests that *M. densirostris* calves disperse from their mothers between 2 -3 years of age, long-term associations with relatively unmarked beaked whale calves are particularly hard to track using photo-identification. In the only study where putative mother-calf relationships were assessed in *H. ampullatus*, repeated associations over two subsequent years were only documented twice (Gowans, 1999; Gowans et al., 2001). From our review, the range of estimates for the duration of lactation, weaning period, age of dispersal or inter-calf interval in beaked whales has either been inferred from the maximum length of maternal-calf associations using photo-identification analysis or applied across species using limited stomach content data (e.g. see New et al., 2013). Thus, our study provides the first significant dataset for interpreting the range of variation in individual maternal investment in a species of beaked whale and improves our understanding of the diversity in maternal strategies found across cetaceans and mammals.

4.7 Acknowledgements

We would like to acknowledge the contributions of the GG Hatch Lab to the analysis of these samples, and support of Dr. Steve Ferguson, DFO. Teeth specimens from whaled animals were contributed by Nils Øien, Institute of Marine Research Norway. Samples from stranded whales were generously contributed by Wayne Ledwell, of the Newfoundland Stranding network.

Chapter 5 Origin and Persistence of Markings in a Long-Term Photo-Identification Dataset Reveal the Threat of Entanglement for Endangered Northern Bottlenose Whales (*Hyperoodon Ampullatus*)

5.1 Publication Status

This chapter was published in Frontiers in Marine Science in April, 2021 as: Feyrer, L. J., Stewart, M., Yeung, J., Soulier, C., & Whitehead, H. (2021). Origin and Persistence of Markings in a Long-Term Photo-Identification Dataset Reveal the Threat of Entanglement for Endangered Northern Bottlenose Whales (*Hyperoodon ampullatus*). Frontiers in Marine Science, 8.

5.2 Abstract

Photo-identification methods depend on markings that are stable over time. Using a large dataset of photographs taken over a 31-year period, we evaluate the reliability, rate of change and demographic trends in different mark types on northern bottlenose whales (*Hyperoodon ampullatus*) in the Endangered Scotian Shelf population, and assess the prevalence and severity of anthropogenically caused markings. Only fin notches and back indentations were stable over long timescales, leading to 48% of the overall population being assessed as reliably marked. Males and mature males were found to have higher incidence of most mark types compared to females and juveniles. The proportion of reliably marked individuals increased over time, a trend that should be accounted for in any temporal analysis of population size using mark-recapture methods. An overall increase in marked individuals may reflect the accumulation of scars on an aging population post whaling. Anthropogenic markings, including probable entanglement and propeller-vessel strike scars, occurred at a steady rate over the study period and were observed on 6.6% of the population. The annual gain rate for all injuries associated with anthropogenic interactions was over five times the annual potential biological removal (PBR) calculated for the endangered population. As entanglement incidents and

propeller-vessel strike injuries are typically undetected in offshore areas, we provide the first minimum estimate of harmful human interactions for northern bottlenose whales. With low observer effort for fisheries across the Canadian Atlantic, photo-identification offers an important line of evidence of the risks faced by this Endangered whale population.

5.3 Introduction

Photo-identification methods are commonly used to identify individual cetaceans using markings of natural or anthropogenic origin, and long-term datasets have revealed valuable scientific information (Ballance, 2018). Critical to investigations of population size and trends (e.g., Barlow et al., 2011; Wilson et al., 1999), scales of residency and ranging behaviour (e.g., Calambokidis et al., 2002; Fearnbach et al., 2014; Gladilina et al., 2018; Mahaffy et al., 2015), demography (e.g., Aschettino et al., 2012), social structure (e.g., Gero et al., 2008), and habitat use (O'Brien et al., 2020), photo-identification has been particularly valuable tool in understanding cetaceans both as individuals and populations. While any distinctive natural markings may be used for individual identification over periods from days to weeks, understanding which markings are permanent or will remain stable over the lifetime of the individual is necessary for reliable long-term identification of individuals. Misidentification due to loss or gain of markings can result in a Type I error (a false positive, incorrectly identifying an animal as a known animal) or Type II error (a false negative, incorrectly identifying a known animal as an unknown or new animal). Long term datasets require regular re-evaluation not only to avoid Type I and II errors, but also to ensure distinctive marks are reliable and do not change or are not lost over the study period (Frasier et al., 2009; Gowans and Whitehead, 2001; Wilson et al., 1999, Urian et al., 2014). Additionally, any trends in the proportion of reliably marked individuals over time could bias population size estimates and need to be incorporated into mark-recapture analyses.

Individual markings, such as distinctive scars or large wounds, can also be used to estimate the prevalence and source of disease or injuries that are natural or anthropogenic in nature, and assess whether there are potential differences within a population (e.g., by age or sex class), over time, or between populations in the rate of predatory or anthropogenic interactions (Baird et al., 2014; Chu & Nieukirk, 1988; Felix et al., 2018). Injuries due to interactions with fisheries (vessels and gear) are thought to be the most important management issue affecting cetaceans (Moore, 2019; Read, 2008). However, with low or no independent observer effort, poor reporting requirements for cetacean bycatch, and limited conclusive necropsies of stranded animals, injurious or fatal interactions of cetaceans with fisheries are especially difficult to quantify (Hines et al., 2020; Van Waerebeek et al., 2007; Williams et al., 2011). The information we do have for many data-poor species is currently limited to bycatch 'anecdotes' (Fisheries and Oceans Canada, 2009; Harris et al., 2013) and screening level risk assessments (e.g., Brown et al., 2013) using broad assumptions about life history, behaviour and habitat. Photographic analyses of scars presumed to be due to interactions with vessels or gear offer valuable information on potential unaccounted sources of cryptic mortality and an opportunity to assess and monitor these anthropogenic impacts on wild populations (Kiszka et al., 2008; Leone et al., 2019, Ramp et al., 2021).

The Scotian Shelf population of northern bottlenose whales (*Hyperoodon ampullatus*) inhabits the deep waters off Nova Scotia and has been extensively studied using photo-identification methods (Gowans and Whitehead, 2001; O'Brien and Whitehead, 2013; Wimmer and Whitehead, 2004). O'Brien and Whitehead's (2013) study found the population was small (~143 individuals), but stable. This population has been designated as Endangered and listed under the Canadian *Species At Risk Act* (*SARA*) in 2006, with associated requirements for protection of critical habitat, ongoing monitoring and recovery measures, and an assessment of current threats (Fisheries and Oceans Canada, 2009). Since commercial whaling for the species ended in 1971, threats to species recovery now include acute injury and mortality from entanglement in fishing gear and ship strikes, as well as chronic and acute threats from noise and ongoing oil and gas exploration (Fisheries and Oceans Canada, 2009; Whitehead & Hooker, 2012). The core habitat for the Scotian Shelf population is centered around the deep waters of the Gully submarine canyon, which was declared as an *Ocean's Act* Marine Protected Area

(MPA) in 2004 (Figure 5.1). While the MPA includes a prohibition against fishing in Zone one, there are no restrictions on fishing activities in the adjacent designated critical habitat areas of Shortland and Haldimand canyons (Fisheries and Oceans Canada, 2009; Figure 5.1).

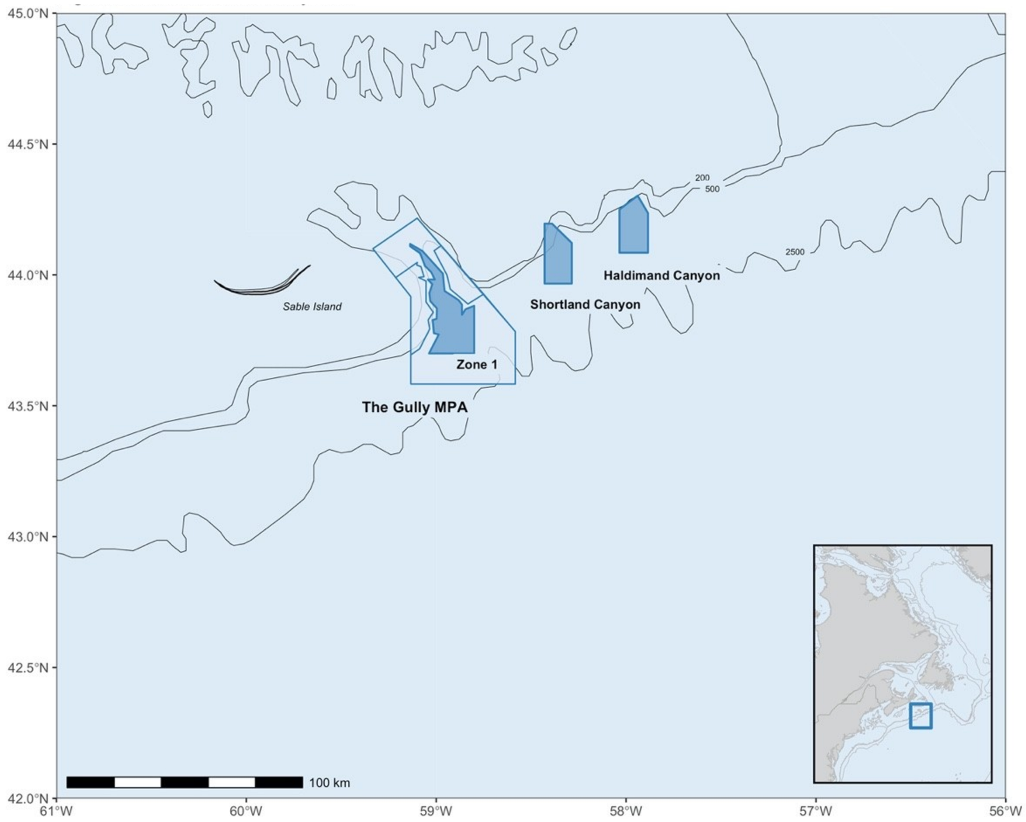


Figure 5.21 Study area extent on the Scotian Shelf. Research over the period 1988-2019 was focussed in the Gully MPA (outlined in blue), particularly the deep waters of Zone one (shaded blue polygon). After 2001 research expanded to include Shortland and Haldimand canyons (shaded blue polygons). These shaded blue areas are also currently designated as critical habitat for northern bottlenose whales.

The impact of acute mortality and injury due to interactions with fisheries on beaked whales, including the northern bottlenose whale, is highly uncertain (Hooker et al., 2019), but the risk has previously been described as low (Brown et al., 2013). Although they are a rarely-seen offshore species, northern bottlenose whales are known to approach boats and interact with fisheries that occur in offshore areas (Fertl & Leatherwood, 1997; COSEWIC, 2011; Mitchell, 1977; Oyarbide Cuervas-Mons, 2008).

However, we are aware of only a few reports of bycaught or gear-entangled individuals in the western North Atlantic over the last 30 years (N = 13, Table 1). Patterns of reported incidents are difficult to interpret as a reflection of temporal trends or risk for a number of reasons. Overall, there seem to be more incidents reported before 2010, and while some areas of the Scotian Shelf have seen a reduction in trawl fishing effort and a ban on drift gill-nets over this period, long-line fisheries in deep water areas have continued. Outside the relatively small area of the Gully MPA's Zone one, long-line fisheries occur along the shelf edge, including in Zone 2 and 3 of the Gully MPA. From the records of entangled beaked whales, we found (Table 5.1) ~46% were attributed to long-line gear, ~23% to trawls and the remaining to other or unknown fisheries (Fisheries and Oceans Canada, 2016; Garrison, 2003; Whitehead et al., 1997). Bycatch records of non-target species brought onboard vessels, which we include here as a source of data on entanglement, suffers from considerable bias in reporting. Due to issues with the spatial representativeness, low levels of observer coverage in the region over the last 30 years (ranging from 0 to 11% of all vessels), the likelihood that large whales are more likely to break free than be brought on board, and variability in the species identification skills of observers, the low number of reports is not informative of the extent or likelihood of beaked whale entanglement incidents (Hooker et al., 1997). Finally, due to their remote habitat, there are few records of beaked whales stranding or washing ashore in Atlantic Canada, and with carcasses in degraded condition and limited resources for forensic investigations, it is typically difficult to attribute cause of death (Benjamins et al., 2011; Lucas & Hooker, 2000; Nemiroff et al., 2010). Despite increased focus on reducing the incidence of entanglement, bycatch, and vessel strikes for other at-risk whale species in Canada (e.g., North Atlantic Right Whale, *Eubalaena glacialis*, Davies & Brillant, 2019; Moore, 2019), there has been limited progress on improving our understanding of the unintended impact of fisheries on beaked whales.

Table 5.17 Records of northern bottlenose whales and other beaked whales caught or entangled in fishing gear in western North Atlantic.

Species	N	Year	Fishery	Description	Location	Reference
H. ampullatus	1	2021	unknown	Gear marks on tail stock and back of stranded adult male.	Newfoundland	Ledwell & Huntington (2021)
H. ampullatus	1	2008-2014	Gear described as "net". Not including additional reports of 4 dead and stranded NBW with unspecified cause of death.	Entanglement resulting in death, from opportunistic reports.	Atlantic Canada	(Themelis et al., 2016)
H. ampullatus	8	1980-2008	Longline gear (n = 3), Trawlers (n = 2), hake/ squid gear ("several")	Serious entanglements by at-sea observers (likely include those reported by Hooker et al., 1997 below).	Scotian Shelf (5), Newfoundland & Labrador (3)	(Fisheries and Oceans Canada, 2009)
H. ampullatus	1	2005	unknown	Gear marks on tail stock of stranded juvenile	Newfoundland	Ledwell & Huntington (2006)
H. ampullatus	1	2003	Longline	Entangled around beak in buoy line, disentangled and released.	Davis Strait	Ledwell & Huntington (2004)
H. ampullatus	1	2001	Longline	Serious entanglement, fatal	Southern Grand Banks, Newfoundland	(Garrison, 2003)
H. ampullatus	1	2001	Longline	Serious entanglement, released	Southern Grand Banks, Newfoundland	pers com reported in Wimmer & Whitehead (2004)
H. ampullatus	1	1999	Longline	Serious entanglement	Gully, Scotian Shelf	(Gowans et al., 2001b)
H. ampullatus	2	1991, 1993	Trawl	Serious entanglement, reported by at-sea observers	East of the Gully, Scotian Shelf	(Hooker et al., 1997)
H. ampullatus	1	pre-2007	Trawl	Decomposed NBW found in trawl reported to fisheries observer	Newfoundland	(Oyarbide Cuervas-Mons, 2008)
M. bidens	2	2013	Line - gear undetermined	Serious entanglement, 1 released by researchers	Gully MPA	(Fisheries and Oceans Canada, 2016)
Other ziphiid sp. *M. bidens* (n=24); *Mesoplodon mirus* (n=4); *Ziphius cavirostris* (n=1); & undifferentiated (n=17)	46	1989-1998	Pelagic drift Gillnet	Mortalities	Bycatch of beaked whales has only occurred from Georges Canyon to Hydrographer Canyon along the continental shelf break and continental slope during July to October.	(NOAA, 2015)

For marine mammals, bycatch, entanglement, and vessel strikes can have both lethal and sub-lethal effects, which, for animals that "survive" may include the associated fitness costs of infection, injury, energetic loss, inability to forage, and reduced reproductive potential (Dolman & Brakes, 2018; van der Hoop et al., 2016; Visser, 1999). While we know interactions with fisheries are fatal for beaked whale species in other areas (Carretta et al., 2008) and are contributing to dramatic declines of endangered marine mammal populations across the globe (Brownell et al., 2019; Moore, 2019; Reeves et al., 2003; Turvey et al., 2007), the impact of this threat on the Scotian Shelf population of northern bottlenose whale is unknown, despite over 30 years of research. However, previous studies examining anthropogenic-caused injuries from scarring in cetaceans have provided insights on the prevalence of their interactions with fisheries (Felix et al., 2018; Kiszka et al., 2008; Leone et al., 2019).

Here we use a large dataset of high-quality identification photographs of northern bottlenose whale dorsal fins and melons from the Scotian Shelf over a 31-year period (1988-2019) to assess the proportion, rate of change, and sex-age class of individuals with natural and anthropogenically-caused markings. Investigating the trends and bias in markings in the population is necessary for robust population estimates, minimizing error rates in identification, identifying the minimum proportion of northern bottlenose whales that have survived an interaction with a fishery or vessel, and estimating the extent of this threat for this species of beaked whales. The objectives of this study were to (1) evaluate the reliability (gain and loss rates) of different distinctive mark types over the 30-year study period and calculate an error rate for misidentifications; (2) assess trends in distinctive mark types occurring in the population over time, before and after the implementation of the Gully MPA, and by sex-age class; and (3) identify the prevalence and severity of anthropogenically-caused scars in the population.

5.4 Methods

5.4.1 Data Collection

The photographic data used in this study were collected during summer field seasons on the Scotian Shelf edge from 1988 to 2019. Photographs were taken of the dorsal area of all northern bottlenose whales encountered, regardless of the presence or severity of markings. The melon (forehead) and both the left and right side of each whale were photographed when possible. Biopsies were collected opportunistically for genetic analysis over this same period using methods described in Feyrer et al. (2019).

5.4.2 Photo-Identification

Previous studies (Gowans & Whitehead, 2001; O'Brien & Whitehead, 2013; Wimmer & Whitehead, 2004) hand matched printed photographs; however, here we compiled digitized versions of previous hard copy catalogues and newer digital photographs using the photographic management software, Adobe Lightroom (Version 6.14; Adobe Inc., 2015) using an updated photo-ID protocol (Feyrer et al., 2021) which is briefly summarized below. The associated metadata for each photograph (e.g., GPS location, quality rating, keywords) and identification information (e.g., sex, ID number) were saved with each digital image and 'collections' were used to track all photographs for each ID. The left and right sides of dorsal fins were considered separately for initial identification and analysis, but when identifiable marks spanned both left and right sides (e.g., a distinctive notch), both ID sides were linked by a common number. Photographs were given a quality rating (Q) based on the angle, focus, visible proportion of dorsal fin, and exposure, similar to criteria used by O'Brien and Whitehead (2013). Poorest quality photographs, which met none or only one of the criteria, were given a rating of Q1, while highest quality photographs, which met all criteria, were rated Q4 (Figure 5.2). The highest quality dorsal fin photographs (left and right side) of each individual identified in each year were put into a type specimen collection. Iterative pairwise comparisons between all type photographs were made within and between years and each individual whale received a unique ID number. The number of IDs, resighting rates, and catalogue years (number of years in the catalogue) were summarized. During the digital compilation of the catalogue, we conducted multiple reviews and validated all Q ratings and previously matched IDs, which allowed us to detect misidentifications and estimate an error rate in matching. Error was calculated as the number of incorrectly matched photographs divided by the number of all ID resights (total number of photographs of all IDs minus their first 'type' sighting photograph) as per Frasier et al. (2009).

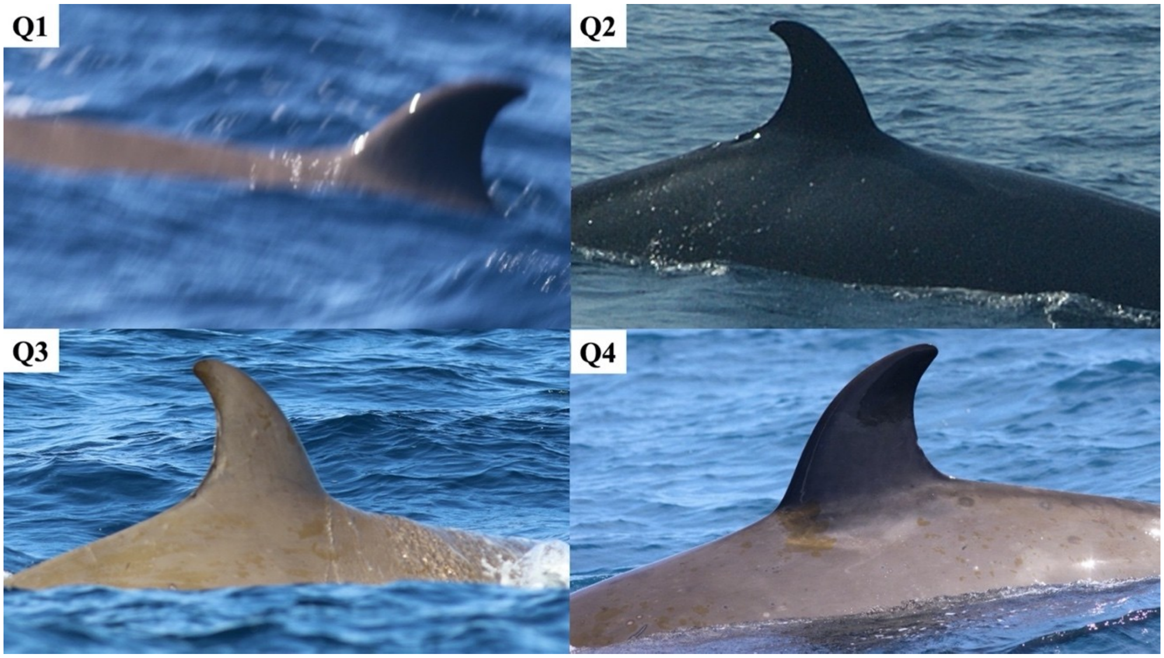


Figure 5.22 Example quality ratings for dorsal fin photographs. Lowest quality photographs are given Q1 and highest quality photographs are given Q4

5.4.3 Melon Age and Sex Analysis

Sex was determined using two methods: (1) genetic analysis of biopsied whales based on Einfeldt et al. (2019) and (2) photographic analysis of the relative "roundness" of melons (foreheads), with males having a square-shaped melon compared to females and juveniles (Gowans et al., 2000; Yeung, 2018). The protocol for sexing northern bottlenose whales using melons has been updated since Gowans et al. (2000) and is based on two classifications: Mature Male (MM) or Female-Juvenile (FJ) (Figure 5.3), omitting the previous third category of Sub-adult Male, due to poor agreement (Type I errors) with paired genetic analyses (Yeung, 2018). Using a separate catalogue of melon photographs that were quality rated, sexed, and linked to high quality (≧ Q3) dorsal fin IDs, we were able to increase the proportion of individuals with sex-age class information based solely on genetic methods from 7% to 44% and the proportion of photos from 25% to 78%. The combined sex-age classes used in all analyses presented here are Male-Mature Male (MMM), which includes both genetic males and IDs with square mature-male melons and Female-Juvenile (FJ), which includes both genetic females and IDs with round FJ melons.

Figure 5.23 Melon profiles of (A) mature males (MM), and (B) female-juvenile (FJ) used in melon sex classification.

5.4.4 Mark Type Classification

Mark type keywords (Table 5.2) were given to all good quality photographs (≧ Q3) in each year, using the best photograph from each year as a guide. To consistently account for differences in the amount of the body visible in each photo, only markings on the dorsal fin or within one fin-width away from the base of the fin, known as the “dorsal skirt” (Figure 5.4), were considered. Markings could be assigned multiple keywords (i.e., entanglement and large body scar) using a modified version of the mark type classification of Gowans and Whitehead (2001; Table 5.2, Figure 5.5A-F).

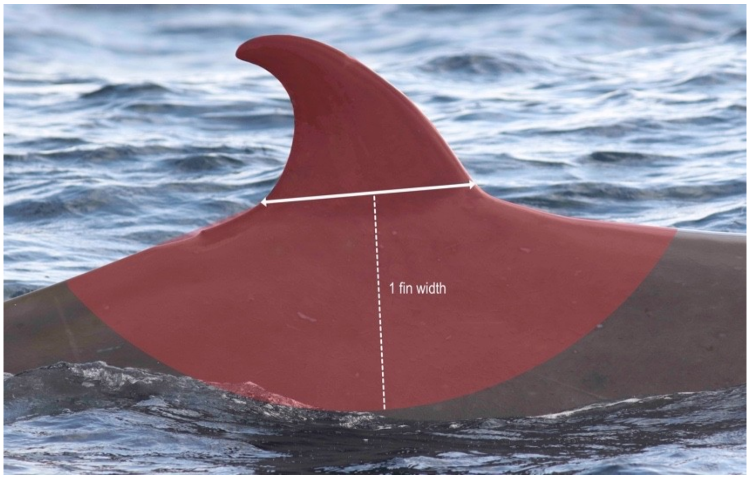


Figure 5.24 The dorsal skirt area where markings and scars were evaluated for reliability are indicated in red shading.

Table 5.18 Mark types used as keywords for identification and matching, adapted from Gowans and Whitehead (2001). Mark types in bold were considered distinctive for individual identification and analysed for rates of mark change in this study.

Mark Type	Description
Back indentation*	Indentation or notch along the spine (below inflection of dorsal fin/spine); Size varies
Circle	Any circular marking(s)
Entanglement	Straight linear furrows that may wrap around the body or crisscross (George et al., 2017)
Large fin scar	Highly identifiable scar on dorsal fin, ≥ 25% of dorsal fin area
Large body scar	Highly identifiable scar on body, occupying at least 25% of dorsal area
LCCS	Lamprey or cookie cutter shark; donut-shaped scar with small teeth marks
Linear	Linear scar, white to grey in colour; Length and thickness varies
Notch*	Where a chunk of fin has been removed (above inflection of dorsal fin/spine); Size varies; White scarring may occur around edges
Patch	Mottled or blotchy patches with soft edges; can be white, grey or black. Size varies; cause(s) unknown.
Propeller	Scar(s) consistent with a propeller strike - large or deep gashes, parallel or “corkscrew” scars (George et al., 2017)
Tooth rake	Two or more parallel linear scars consistent with teeth spacing of other odontocetes.
Slough skin	Light discolouration in irregular angular shapes from the peeling off of skin; changes rapidly (within days), not used for identification.
Clean	Having none of the mark types listed above.

* indicates reliable marks.

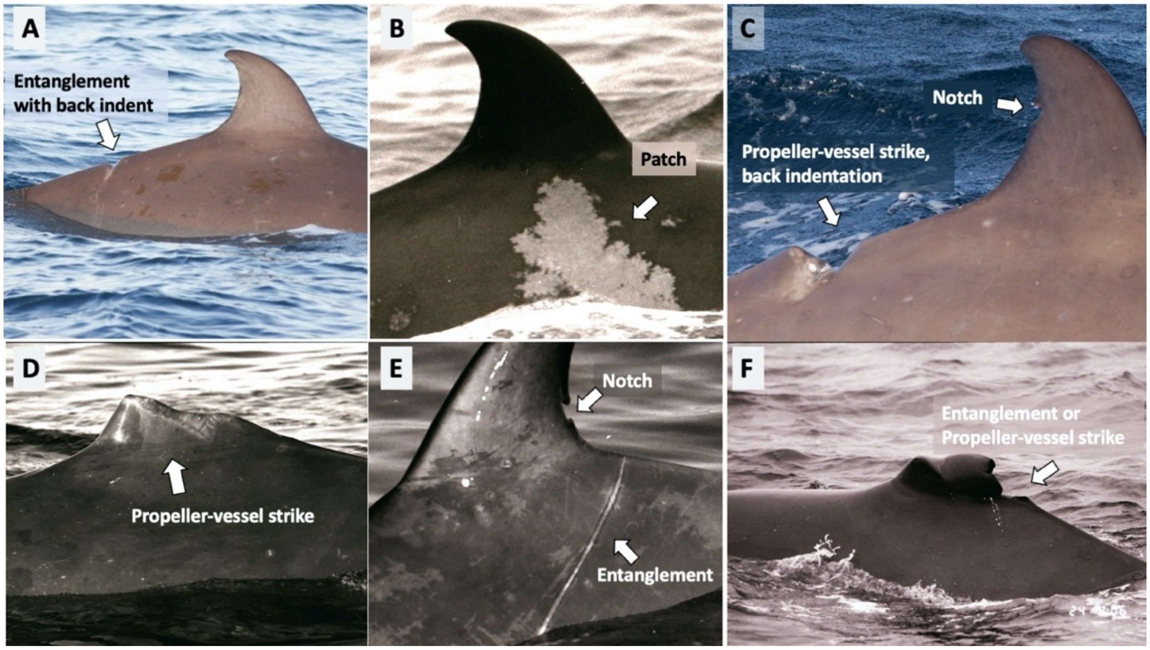


Figure 5.25 Examples of mark types and keywords, as annotated on dorsal fins of northern bottlenose whales. Alternative keywords are provided in parentheses. A. Back indent, entanglement; B. Patch (large body scar); C. Propeller-vessel strike, back indentation, notch (large body scar); D. Propeller-vessel strike, (large fin scar); E. Notch, entanglement (large body scar); F. Entanglement, propeller-vessel strike (large fin scar).

Anthropogenic markings, specifically those caused by injuries related to entanglement or propeller-vessel strikes, have not previously been described in northern bottlenose whales. However, observations of entangled or bycaught northern bottlenose whales (Table 5.1) as well as photographic evidence of actively entangled Sowerby's beaked whales (*Mesoplodon bidens*) in the Gully MPA in 2013 (Figure 5.6A,B) and northern bottlenose whales in the Gully in 1990 and Davis Strait in 2003 (Figure 5.6C,D) indicate that these threats do occur at some level. The literature on cetacean entanglement and ship strikes provides a wealth of descriptions and well-documented images of multiple species with scars from entanglement or propeller-vessel strikes on tail flukes, peduncles, dorsal fins and backs that can be used as reference points for comparative analysis (e.g. George et al., 2017; Basran et al., 2019; Felix et al., 2018; Baird et al., 2014; Kügler & Orbach, 2014; Visser, 1999). We initially classified anthropogenic marks based on (1) the features of entanglement and propeller-vessel strike scars documented and described in other studies and (2) our analysis of scarring resulting from entanglement injuries observed on live beaked whales (Figure 5.6), gear marks on dead stranded northern bottlenose whales (unpublished data, Ledwell & Huntington, 2005), and a video of an entangled northern bottlenose whale recorded in the Gully (Whitehead Lab, 1999). IDs with anthropogenic marks were then reviewed by external experts with experience in large whale entanglement, beaked whales and gear used in the region's offshore fisheries. Reviewers ranked images of each possible ID on a scale of 1-3 with 1 being low confidence and 3 being high confidence that marks were probable entanglement or vessel strike and only those IDs which reviewers agreed with high confidence were included in further assessment of anthropogenic marks.

In our initial review of the patterns of tissue damage and scarring seen in the dorsal fin region, we screened the dataset multiple times for possible anthropogenic markings. The identification of entanglement marks used in our assessment included a range of scars that can be caused by the rubbing or pressure of a rope or line as it is wrapped around the body, fin or tail stock of an animal, and these scars are typically characterized by the presentation of a curvilinear pattern of relatively consistent thickness and tapering ends (see George et al., 2017; Basran et al., 2019; Robbins & Mattila, 2001). However, during the process of entanglement, the haphazard wrapping, knots and criss-crossing of various types of fishing gear, can blend, abruptly break or change the direction of the linear pattern of scarring (Robbins, 2009; Figure 5.6). The weight, tension or restricted movement of entanglement can cause lines to become deeply embedded and result in deep spine indentations (Robbins, 2009), fin mutilations (Baird et al., 2014) or protruding scar tissue (see Figure 5.6). Examples of severe entanglement injuries on a beaked whale body shape were key references in our analysis and are presented in Figure 5.6. The individual in Figure 5.6A was a Sowerby's beaked whale with multiple curvilinear scars from an entanglement in a line forward of the dorsal fin; one wrap of the line appears to be still embedded in the animal's flesh, causing raised tissue and possible necrosis. The curvilinear scars are of consistent thickness until they taper where broken, likely caused by the raised spinal processes and inward curvature of the animal's poor body condition or shifting lines. A second Sowerby's beaked whale (Figure 5.6B) has a rope tightly entangled around its body and pectoral fin, causing deep lacerations into the blubber layer. The location of the entanglement likely restricts movement, and while the rope has become embedded in the animal’s flesh, where the line does not have contact with the skin there are again breaks in the scar pattern where the skin tissue is still intact. Adjacent to the embedded line there are non-linear areas of abrasion, possibly due to the chafe from a previous positioning of the embedded line or a secondary line that was lost. The individual in Figure 5.6C is a live northern bottlenose whale that was entangled in the Davis Strait in 2003 with a longline buoy line wrapped around its beak, while the animal was calm and later released, the linear abrasions around the beak blend together, and blood is coming from the mouth near the wrap point of the line. Figure 5.6D is a male northern bottlenose whale photographed in the Gully in 1990 with multiple wrapping scars around its body, well forward of the dorsal fin and behind the melon. While no scaring is apparent in the region of the dorsal fin, and this individual is not included in the analysis of dorsal fins we present, the scars appear to blur together with varying thicknesses, angles, and severity, with some lines ending abruptly. The last reference we used was a video taken in 1999 of a northern bottlenose whale in the Gully with a monofilament line wrapped around its beak, possibly hooked in its jaw (Whitehead Lab, 1999). It appears to be resting with its head and beak at the surface, and both are clearly scarred with a thin wrapping diagonal white line going over the left side of the melon and across the blow hole, however this scar line does not appear to continue on the right side of the animal (Whitehead Lab, 1999).

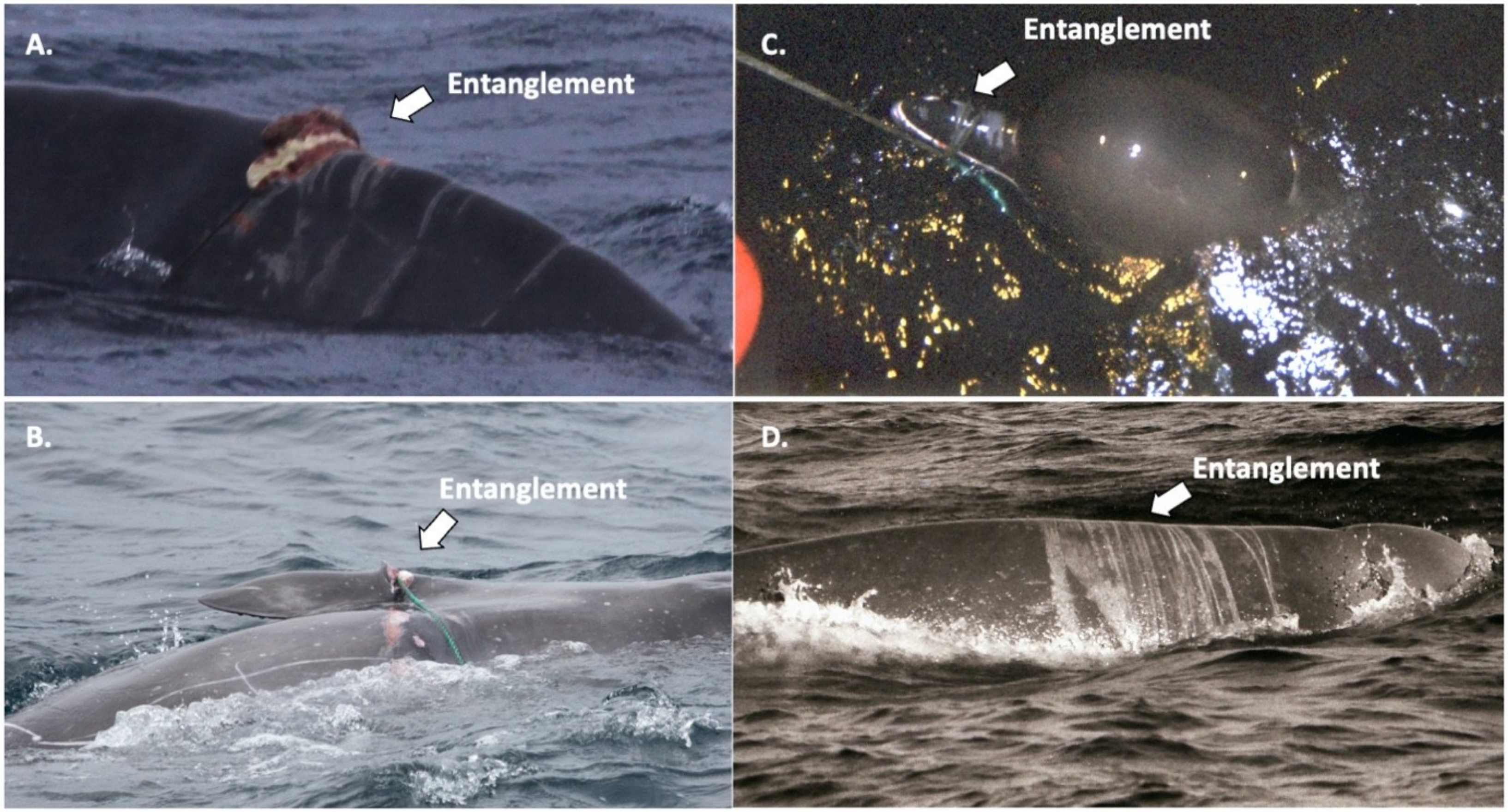


Figure 5.26 Examples of entanglement used for comparative analysis of scar patterns (A,B) depict two different entangled Sowerby's beaked whales observed in the Gully MPA in 2013; (C) a northern bottlenose whale entangled in the Davis Strait in 2003 with a longline buoy line wrapped around its beak; and (D) a male northern bottlenose whale in the Gully in 1990 with multiple wrapping scars around its body.

Injuries related to vessel strike incidents have been well characterized in large whales as resulting in: (1) blunt force trauma causing significant fractures, but potentially few other externally apparent injuries (Vanderlaan & Taggart, 2007; Laist et al., 2001); and (2) propeller wounds, which include deep slashes or indentations (Laist et al., 2001; Van Waerebeek et al., 2007; Visser, 1999), mutilated or chopped dorsal fins (Van Waerebeek et al., 2007), and parallel concave lacerations (George et al., 2017). However, severe entanglement can also result in fin mutilation or amputation, and it is not always possible to conclusively attribute propeller-vessel strike as the source of less severe injuries. As a result, all marks initially attributed to either probable entanglement (Figure 5.5A,E,F) or vessel-propeller strike injuries (Figure 5.5C,D,F) were combined into one category for anthropogenic scars (Moore et al., 2013). Based on the severity of injury, we also classified anthropogenic scars using a qualitative three-point scale with 1 being low severity and 3 being most severe (Appendix D, Figure D1).

5.4.5 Mark Type Analyses

Mark types selected for analyses included notches, back indentations, large dorsal fin scars, patches, and anthropogenic scars as described above and in Table 2. These markings were selected as they are highly distinctive (Urian et al., 2014), most commonly used for inter-annual identification, and determining their prevalence, longevity and reliability has important implications for mark-recapture population analyses, as well as our understanding of potential threats to the population (Fisheries and Oceans Canada, 2009; Gowans & Whitehead, 2001; O'Brien & Whitehead, 2013; Table 2). For each mark type, we assessed all IDs that had at least one high quality photograph with the mark keyword. Mark type classifications were not mutually exclusive, as notches or back indents were in some cases also assessed as anthropogenic (see Figure 5), but all marks were analysed separately.

5.4.5.1 Prevalence

The prevalence of the different mark types in the population was calculated separately for left and right IDs and averaged across data collection years. We used binomial generalized linear regression models (GLMs) to assess whether the proportion of marked individuals (right and left side catalogues calculated separately) had either (a) increased, (b) remained stable or (c) differed between the years occurring prior to or after the MPA. We determined the best fit trend based on lowest AIC (Akaike's information criterion) score, with all models having scores ΔAIC < 2 considered as demonstrating some support. The relationship between the proportion of marked IDs in MMM and FJ sex-age classes and the proportion of marked IDs where we have genetic information on molecular sex (XY males and XX females) was tested using linear regression. For each year where there were > 10 IDs, we compared the difference between the proportion of MMM and FJ sex-age classes using paired t-tests. All data were normally distributed across years. Statistical analysis was completed in MatLab (2019) and R (2019).

5.4.5.2 Change

Annual rates of loss or gain were analysed separately for each mark type for all IDs seen in multiple years. For each year that an ID was in the catalogue, a change was recorded as either negative (a decrease in the visible mark size or number), positive (an increase in the visible mark size or number), or none (no change in the mark size or number). If an ID entered the catalogue with a mark, nothing was recorded until a change occurred, and if gains (or losses) occurred they were counted once in the first year they were observed. The most recent photograph was always used to compare between subsequent years, and only the highest quality photographs (Q4) were used to analyse mark change for patches, large scars and anthropogenic marks, while analysis of back indentations and notches also used photographs of good-excellent quality (≧ Q3). The average rate of change was calculated separately for each mark type, summing total gains or losses and dividing by the total whale years for all reliably marked individuals in the catalogue. Total whale years are defined as the number of years an individual appears in the catalogue (i.e., year of last sighting – year of first sighting), and rates were calculated as per Auger-Méthé and Whitehead (2007):

(1) Rate of gain = Total number of gains / total whale years

(2) Rate of loss = Total number of losses / available whale years with marks

Gowans et al. (2001) considered marks reliable for re-identification if they had a zero rate of loss in more than five individuals. Due to the larger scale of this analysis, here we define a mark as reliable if loss occurred less than once in a hundred whale years. Using our definition of reliability, the rate of change in status from unreliable to reliable was calculated for all IDs and years. To estimate the number of whales per year that acquire anthropogenic injuries, we multiplied the most recent published population estimate for the Scotian Shelf (~143 individuals, O'Brien & Whitehead, 2013) by the annual gain rate calculated for probable entanglement and propeller vessel-strike scars.

5.5 Results

5.5.1 Photo-Identification Catalogue

The Scotian Shelf northern bottlenose whale catalogue contained 29,529 dorsal and 9,000 melon jpeg images from 280 days of fieldwork in 25 years between 1989 and 2019. The sample sizes for photographs and identifications for left and right sides are detailed by year in Appendix D (Table D1). Quality rating was reviewed for consistency across years and, due to the effect of low-quality photographs on resighting rates (Urian et al., 2014), photographs < Q3 were not included in the analysis. The catalogue contained 662 right side and 677 left side individuals, with an overall average discovery rate of 28 new identifications per year (but only 8.5 reliable IDs per year, Figure 5.7). Of all IDs, only 33% were seen in a subsequent year; however, for reliably marked whales, 60% of IDs were seen in more than one year, not including IDs first sighted in 2019, the last year in the catalogue (Appendix D, Figure D2). For individuals seen across multiple years, the average number of sighting years was 3.65 (SD = 2.35), with a maximum of 17 sighting years out of a possible 25 years of data collection. A small group of IDs (n = 15) had resights spanning 25-30 years of the 31-year study period.

The error rate for ID matching in previous studies of the population (Gowans & Whitehead, 2001; O'Brien & Whitehead, 2013; Wimmer & Whitehead, 2004) was estimated to be 3.6%. Photo-identification errors that were detected during validation (N

= 1025 photographs) were largely (78%) duplicates (i.e., Type II, false negatives) with only 22% misidentifications (Type I, false positive errors). All IDs were noted and corrected. Of the 131 IDs with photographs affected by errors, nearly 15% (N = 19) had acquired a notch or back indent during the thirty-one year study period, significantly changing their appearance.

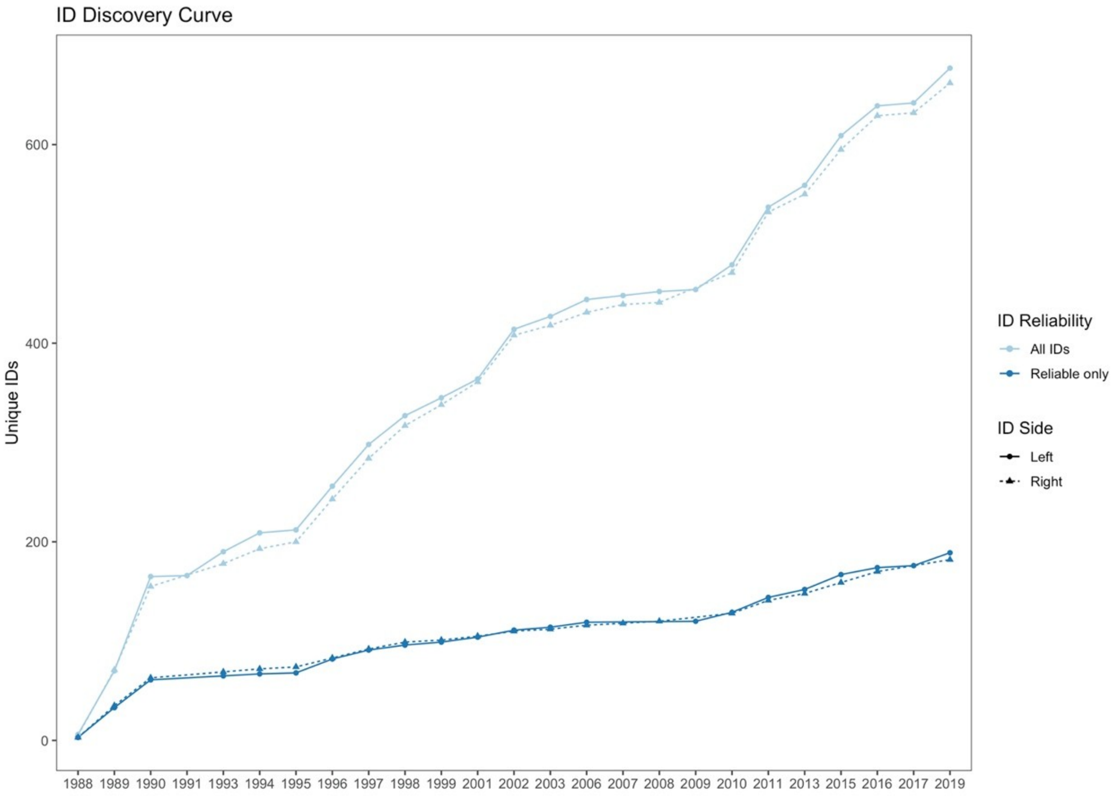


Figure 5.27 Cumulative discovery curve of all unique IDs (light blue line) and reliable IDs only (dark blue line), plotted for left and right sides, 1988-2019.

5.5.2 Mark Prevalence

In the catalogue, 45.3% (SE 1.2%) of all individuals had a notch in their dorsal fin, patches were the next most common mark type with 17.4% (SE 1.4%) of IDs, and other marks occurred in less than 10% of IDs (Table 5.3). Approximately 35% of all IDs were "clean", having none of the distinctive mark types analysed here (Table 5.2). The prevalence of each of the five mark types was similar whether melon or molecular sex classifications were used to identify sex ($R^2 = 0.944$, $P = 0.001$), suggesting that regardless of age, males were generally more marked than females (Appendix D, Table D2). In paired *t*-tests for each year (df = 17), MMM were significantly more scarred (5-20%) than FJ in each mark type category, except for patches, where the proportion of FJ was 7% higher than MMM ($p = 0.011$, Table 5.3). An increasing trend in prevalence was well supported ($\Delta AIC < 2$) for most marks, but for large fin scars and anthropogenic scars a stable trend was the best supported model ($\Delta AIC = 0$). The effect of MPA had some support in comparison of candidate models for indent, large fin scar, and anthropogenic mark types, however, there was stronger support for stable or increasing trends (Table 5.4, Figure 5.8A-G). Most mark types, with the exception of large fin scars, appeared more prevalent in the period after the 2004 designation of the Gully MPA (Table 5.3).

Table 5.19 Average proportion of marked IDs 1988-2019. Presented as an overall percentage, ± standard error with the total number of marked right + left sides (n) over all years and for both sex-age classes. For marked IDs with sex-age class information*, the proportion of marked to unmarked Males-Mature Males (MMM) and marked to unmarked Female-Juveniles (FJ) in each year with > 10 IDs were compared using a paired t-test. The proportion of marked IDs between pre MPA (1988-2004) and post MPA (2005-2019) time periods is presented, although the effect of MPA was not well supported in GLM analysis.

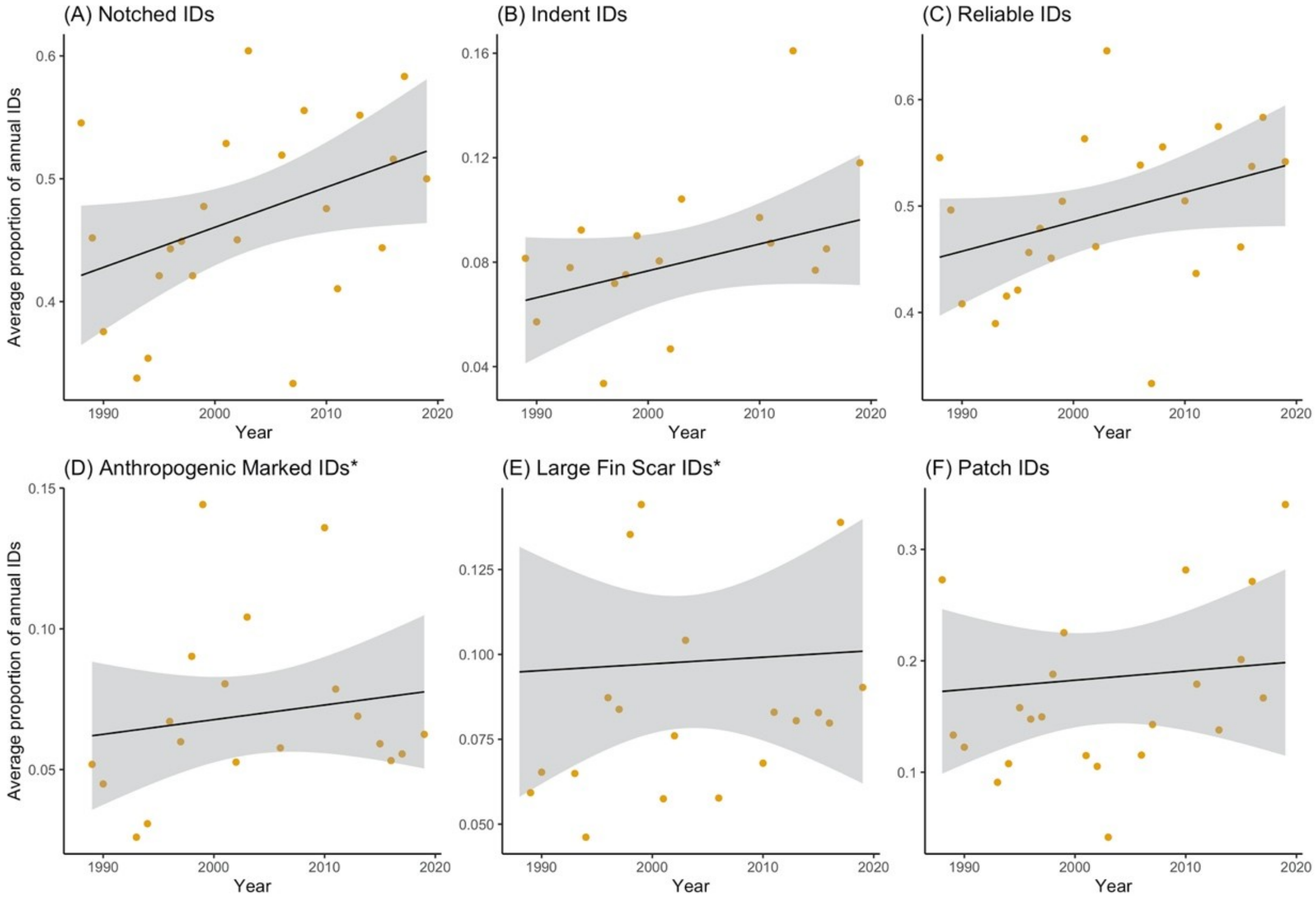
Mark Type	**Total % ±SE (n)**	**MMM % ±SE (n)**	**FJ % ±SE (n)**	**Paired *t*-test MMM *vs.* FJ df = 17**	**Pre-MPA % (n)**	**Post-MPA % (n)**
Notches**	45.3 ±1.0 (1114)	71.0 ±2.0 (475)	46.3 ±1.6 (428)	$t = 6.38$ ($p < 0.001$)	43.5 (618)	47.7 (496)
Back indents**	7.6 ±1.0 (186)	15.7 ±1.1 (105)	5.1 ±0.9 (47)	$t = 6.85$ ($p < 0.001$)	6.6 (94)	8.8 (92)
Large fin scars	8.3 ±1.0 (203)	12.1 ±1.0 (81)	8.1 ±1.0 (75)	$t = 2.22$ ($p = 0.039$)	8.3 (118)	8.2 (85)
Patches	17.4 ±1.0 (427)	14.8 ±0.01 (99)	22.1 ±1.8 (204)	$t = -2.82$ ($p = 0.011$)	13.8 (196)	22.2 (231)
Anthropogenic (entanglement or propeller) scars	6.6±0.5 (163)	14.9 ±1.2 (100)	5.3 ±0.6 (49)	$t = 7.62$ ($p < 0.001$)	6.4 (91)	6.9 (72)

*Sex-age class was known for 64% notches, 63% back indents, 64% of dorsal scars, 56% patches, 92.5% anthropogenic scars

** Indicates reliable marks.

Table 5.20 Summary of binomial generalized linear regression models (GLMs) used to assess whether the proportion of marked individuals had either (a) remained stable, (b) differed between years occurring prior to or after the MPA or (c) increased over time (1988-2019). Right and left side catalogues were calculated separately, results presented use identifications from both the left and right sides. Greatest support is indicated by lowest ΔAIC (Akaike's information criterion) values; all model with ΔAIC < 2 are indicated in bold. Mark types with support for significant increase over time (p-value <0.05) are noted*. The year 2004 was used as a midpoint for MPA trend analysis.

Mark Type	**Stable trend**		**MPA trend**		**Increasing trend**				
	AIC	ΔAIC	AIC	ΔAIC	AIC	ΔAIC	Trend est.	SE	p-value
Notch*	151.21	7.68	149.00	5.47	143.53	0.00	0.013	0.005	0.009
Indent*	110.99	3.47	108.78	1.26	107.52	0.00	0.018	0.008	0.035
Large fin scar	102.43	0.00	104.42	1.99	103.89	1.46	0.006	0.007	0.416
Patch*	180.66	32.32	153.41	5.07	148.35	0.00	0.032	0.008	0.001
Anthropogenic - Entanglement / propeller scars	107.94	0.00	109.69	1.74	108.80	0.85	0.009	0.009	0.381
Reliable*	148.94	5.39	147.92	4.36	143.56	0.00	0.011	0.005	0.020



(A) Notched IDs

(B) Indent IDs

(C) Reliable IDs

(D) Anthropogenic Marked IDs*

(E) Large Fin Scar IDs*

(F) Patch IDs

Figure 5.28 Annual proportion of marked individuals (all ID sides) 1988-2019 for (A) notches, (B) back indents, (C) all reliable marks, (D) anthropogenic scars, (E) large fin scars, and (F) patches. Black line is GLM trend for average proportion by year, gray shading indicates

standard error. *Note that GLMs for anthropogenic or fin scar mark types did not show strong support for an increasing trend. Scale of y-axis differs between mark type trend plots.

5.5.3 Rate of Mark Change

Marks with the highest average rate of gain were notches (8.2%), but had a very low rate of loss (0.2%) per year. Marks with high gain and loss rates were patches (3.1% gain, 6.3% loss per year) and large fin scars (2.1% gain, 10.8% loss per year; Table 5.5). Back indents were found to have a low rate of mark gain (0.7% per year), and no mark loss (Table 5.5). The gain rate for anthropogenic marks was 1.2% per year, with higher rates of mark loss (3.3% per year).

5.5.4 Reliability

Over the 31-year study period, only notches and back indents had low enough loss rates to be considered reliable, resulting in an average proportion of 0.479 (SE = 0.013) IDs that were reliably marked. Of the IDs seen in multiple years, 24 changed status to reliable during the study period, with an annual rate of change of 1.6%. Over time, the proportion of reliable individuals increased at 0.011 per yr (P = 0.02) (Table 5.4).

Table 5.21 Annual rates of mark change found on the Scotian Shelf 1988-2019. All ID-sides with > 1 year of high-quality (Q4) photographs with marks were analyzed for mark change. Rates of gain were estimated for each mark type by dividing the observed number of gains by the total number of whale years in the catalogue. Rates of loss were estimated based on the observed number of losses per mark type, divided by the total number of years where whales were observed with the mark, which varied by mark type.

Mark Type	**Analyzed ID sides (Total Whale years)**			**Rates per year (95% CI)**	
	Total	**Gains**	**Losses**	**Gain**	**Loss**
Notch	249	122 (1494)	3 (1258)	0.082 (0.068 – 0.097)	0.002 (0.00 – 0.007)
Back indent	44	10 (1494)	0 (252)	0.007 (0.003 – 0.012)	0.000
Large fin scar	58	32 (1494)	20 (185)	0.021 (0.01 – 0.030)	0.108 (0.07– 0.162)
Patches	115	46 (1494)	34 (541)	0.031 (0.022 – 0.041)	0.063 (0.044 – 0.087)
Anthropogenic entanglement / propeller scars	43	18 (1494)	8 (243)	0.012 (0.007 – 0.019)	0.033 (0.014 – 0.063)

5.5.5 Anthropogenic markings

Within the catalogue, 6.6% of IDs (with photos >Q3) had one visible clear scar of probable anthropogenic origin (classified as either entanglement or propeller-vessel strike scars). Of the 54 IDs (left and right side combined), 43 IDs or ~80% were seen in more than one year, allowing us to calculate the rate of mark gain and loss. With a population size of 143 individuals (O'Brien & Whitehead, 2013), the estimates of annual gain rate equate to ~1.72 whales per year gaining injuries related to entanglement or propeller-vessel strikes. In qualitative review of scar severity, we found the majority of anthropogenic scars (57%) were considered low – moderate severity (Level 1- 2) and 16% were considered severe injuries (Level 3) such as mutilations or amputations. Most of the scars initially classified as propeller-vessel strike scars were by definition moderate-high severity injuries (Level 2-3); however, external reviewers indicated many of these scars could also have been caused by severe entanglement.

5.6 Discussion

5.6.1 Challenging Assumptions and Testing Hypotheses with Long-Term Data Sets

Long-term field studies of cetacean populations, such as the Scotian Shelf northern bottlenose whales, have generated detailed photo-identification datasets which have become an important resource for species management. With a large and growing catalogue of northern bottlenose whales, researchers have been able to answer questions and provide new data on population status, demographic differences, movement, social structure, and threats (Gowans et al., 2001; O'Brien & Whitehead, 2013; Wimmer & Whitehead, 2004), vastly improving our understanding of the status of this enigmatic and difficult to study species. Over the last 31 years, researchers have identified hundreds of individual northern bottlenose whales, some of whom have been seen repeatedly in the study area from 1988-2019, suggesting they are close to the 37-year minimum estimate of life expectancy currently understood for the species (Christensen, 1973). These long-lived individuals represent the first generation of northern bottlenose whales to be born into the post-whaling period (i.e. after 1972, Whitehead & Hooker, 2012), living through a new era of industrial exploration and the implementation of the first offshore MPA in Canada. While the Scotian Shelf photo-identification dataset is critical for estimating the size of the Endangered population and understanding their status, ongoing monitoring of individuals can be used to improve our appreciation of northern bottlenose whale life expectancy, population age structure, ontogenetic development, and potential changes in patterns of site fidelity in the study area.

Our initial interest in looking at the occurrence of marks over time was to see whether we could detect a change in the proportion of marked individuals after the implementation of the Gully MPA in 2004. While the effect of time was not strong, there was a significant increase in the proportion of individuals with notches and patches over the entire study period. This increase in the proportion of marked individuals could reflect a post-whaling demographic shift in the age distribution towards older individuals, which tend to be more marked, as whaling in the 1960's removed a substantial proportion of the population (Whitehead & Hooker, 2012). As we are unclear on the etiology of notches and patches, their prevalence could also represent a novel pathogen or parasite, an increase in interactions with predators, anthropogenic activities, or even sex-biased migration between areas (Bossley & Woolfall, 2014; Hamilton & Marx, 2005; Wilson et al., 2000). Despite our optimistic hypothesis, it is not entirely surprising that the Gully MPA, which only restricts fishing and vessel traffic in a small deep-water area (475 km^2) of Zone one, has not had a measurable effect on the proportion of marked individuals in the population. Scotian Shelf northern bottlenose whales regularly travel outside the protected area of the Gully MPA and can be found in Shortland and Haldimand canyons where there are few restrictions on human activities (Figure 5.1; Wimmer & Whitehead, 2004). While little is known about migratory movements between the Scotian Shelf and other populations, the distribution of acoustic detections along the shelf edge (Feyrer, unpublished data) between the Gully and the foraging aggregation recently discovered off of Newfoundland (Feyrer et al., 2019) suggests individuals may make longer distance movements from regions where fishing activities are less restricted. Examining differences in the proportion of marked IDs between regions could shed light on geographic differences in the origin of certain mark types and improve our understanding of connectivity across the species range. Further study is required to understand the relative contribution and significance of these potential sources, and whether an overall increase in marks becomes a long-term trend in the Scotian Shelf population.

In modernizing the historically printed catalogue to a digital database, we updated matching and quality rating protocols as per best practises recommended by Urian et al. (2014). Through this process we were able to detect and correct mistakes, and estimate the identification error rate for the catalogue, which suggests it is low and in line with error rates found in other cetacean studies (3.09%, Frasier et al., 2009; 3.38%, Stevick et al., 2001). Duplicate IDs represented the majority of errors, which is typical of protocols that require multiple reviewers to confirm a match as they can more easily screen against false positives (Urian et al., 2014). In our protocol we were able to detect duplicates by having a single technician dedicated to the time-consuming task of reviewing all previous matches. While having one consistent reviewer was useful for standardization across years, with ~450,000 pairwise matches per side, it is unrealistic as the catalogue continues to grow and individuals with knowledge of the IDs in the catalogue leave the project. The ~8.9% combined gain rate for reliable marks and a change in reliability status of 1.6% per year, suggests that new identifications of reliable individuals should be carefully evaluated due to the risk of duplicates and that reliability trends, although small, should be incorporated into population size estimates and monitored on an ongoing basis in long-term datasets (Urian et al., 2014). In the future, automated identification software and further classification of individual distinctiveness, such as refining the definition of notches or unusual scars by size or uniqueness, could help minimize pairwise matching requirements, reduce errors, and increase confidence in population estimates (Hupman et al., 2018).

5.6.2 Demographic Differences

In this study, we found significant differences between the proportion of the MMM and FJ age class in every mark type category, similar to Gowans and Whitehead (2001) who found mature males had significantly more reliable marks than female-juveniles. A lack of repigmentation in scars has been hypothesized to serve as a social signalling function in some cetaceans and, in beaked whales, males typically become more scarred with age, presumably due to male-male competition (Hartman et al., 2015; MacLeod, 1998). While we did not analyse the extent of tooth rake scars, northern bottlenose whales are different from other odontocetes and even other beaked whales, in that they only have two small teeth (< 4 cm average total length), which only occur in mature males (> 15 years) and barely extend beyond the gums at the front of the jaw (Gol'din, 2014; Christensen, 1973; Feyrer, unpublished data). Although male northern bottlenose whales have been described to engage in "head-butting" (Gowans and Rendell, 1999), due to a lack of dental weaponry, we think conspecific interactions are unlikely to cause deep or significant scarring in this species. Scar patterns of multiple parallel lines most likely originate from interactions with toothier species, such as dolphins or pilot whales (*Globicephala melas*). The higher proportion of MMM IDs with more severe reliable marks (notches and back indents) and anthropogenic scars may be linked to a higher risk tolerance in males (Altmann, 1958; Frid & Dill, 2002; Symons et al., 2014), resulting in additional interactions with predators, vessels or large debris. Although FJ have proportionally more patches than MMM, due to the temporary nature and variable size and shape of patches, there is much we don't understand about this mark type. Given the small effect size in this difference and the inclusion of juvenile males within the FJ sex-age class, there is still some uncertainty whether sex or age is most relevant. Even within our large long-term dataset, there are few female IDs that can be classified as mature based on their sighting history, limiting our ability to separate the effect of sex and age for females.

While we did not attempt to assess how the age of individuals affects the proportion of injuries, it is possible that life history stage, which is poorly known within the population but may have shifted since whaling ceased, is potentially confounding the assessment of change over time. Generally, calves and juveniles are less marked than mature individuals due to exposure time, while older whales may be more experienced or large enough to survive interactions with predators and break free from fishing gear

(George et al., 2017). A juvenile northern bottlenose whale observed by the Whitehead Lab (1999) with an active monofilament line entanglement around its beak was thought unlikely to survive, which suggests another potential bias in any estimates of the rate of anthropogenic interactions by age class. However, the relationship between mark type occurrence and life history stage has previously been used to assign age-classes to other cetaceans (Hartman et al., 2015) and is another area for research in this species.

5.6.3 Mark Change and Reliability

In cetacean species that do not present natural variation in pigmentation, fin, or fluke profile, individuals can only be reliably identified by the irregular occurrence and persistence of scars from parasites, disease, interactions with predators, conspecifics or anthropogenic activities. However, scar pigmentation and accumulation vary widely across cetacean species, with scars persisting for the lifetime of an animal (e.g., Rissos's dolphins, *Grampus griseus,* Hartman et al., 2015) or fading within months to a few years (e.g., common bottlenose dolphins, *Tursiops truncatus*, MacLeod, 1998). As long-term photo-identification studies are a primary source of information on cetacean population status and trends, there needs to be a clear understanding of the reliability and rate of change in marks used to match individuals and scale population estimates (Frasier et al., 2009; Hupman et al., 2018). As mark loss violates the assumptions of mark-recapture analysis, only marks that have been analysed for reliability at the scale of the period under consideration should be used. Here, the only scars that met the criteria for long-term reliability in northern bottlenose whales were fin notches and back indents, which persisted over multiple years with low to zero rates of mark loss. Although patches were considered "reliable" over the nine-year period analyzed by Gowans and Whitehead (2001), with additional years and repeat sampling events, we determined that this mark type may be distinctive, but is not stable due to high rates of loss. Omitting patches as reliable marks reduces the proportion of IDs considered for population estimation from 66% (Gowans & Whitehead, 2001) to 49.8% (this study), but their inclusion may have inflated mortality rates of previous population estimates for this species, as individuals that lose marks may be lost from the record and considered (by the mark-recapture analyses) as probable mortalities (e.g., the estimated mortality was 11% in O'Brien & Whitehead, 2013). Combinations of distinct but non-reliable mark types are still useful for matching individuals within a season or between adjacent years, however, without distinctive mark types (35% of IDs were considered "clean"), repeat identification within the long-term dataset becomes unlikely and is a source of error. Fin shape classification, which has been looked at in other species (e.g., blue whales, *Balaenoptera musculus,* Gendron & Ugalde de la Cruz, 2012)*,* may help further distinguish "clean" and other poorly marked individuals; however, fin shape can be distorted in lower quality photographs and relies heavily on photographs having a consistent angle to the body's position and roll for comparison.

5.6.4 Implications of Anthropogenic Interactions

In addition to unnatural levels of mortality, there are other population level impacts from the short-term stress of an entanglement or vessel strike incident, including long-term energetic costs that may reduce a survivor's reproductive output (van der Hoop et al., 2016). Baird et al. (2014) found female false killer whales (*Pseudorca crassidens*) were more likely to bear scars from interacting with fisheries, with potentially significant impacts to reproductive capacity and calf mortality. While we found the MMM age class of northern bottlenose whales were more likely to possess anthropogenic scars, we do not know the sex or age of all marked IDs. In addition, the relationship between scarring and mortality, which may favour the survival of larger or older animals, limits our understanding and interpretation of population level impacts. While the majority of probable entanglements left scars of low severity, we did not assess entanglement scarring in other areas (e.g., the beak or tail fluke), which have been observed in northern bottlenose whales and found to be more prevalent or serious than those of the dorsal fin area in other species (Fisheries and Oceans Canada, 2009; Whitehead Lab, 1999; Whitehead et al., 1997; Ramp et al., 2021). Although the mortality of vessel strike injuries in cetaceans is generally assumed to be quite high, blunt force trauma is harder to detect than mutilations (Laist et al., 2001; Vanderlaan & Taggart, 2007). However, mutilations related to severe entanglement or propeller vessel strikes are hard to distinguish, leading us to combine our assessment of the scars we attribute to probable anthropogenic sources. Overall, we found 6.6% of the population had experienced probable entanglement or propeller-vessel strike scars in the region of their dorsal fin. Our analysis of mark rates over this period suggests that on average 1.72 whales per year suffer injuries related to probable entanglement and propeller-vessel strike combined. This rate of anthropogenic interactions is of concern as it is over 5 times the potential biological removal (PBR) of 0.3 individuals per year estimated by Harris et al. (2013). Although PBR generally refers to removals due to mortality events, we use it here as a threshold for comparison because (1) many non-fatal anthropogenic injuries may eventually result in mortality, (2) injuries can have long-term impacts to the reproductive capacity of individuals, which would limit population growth, and (3) the rate combined with the risks associated with interactions suggests that there are an unknown number of individuals in the population that do not survive. Taken altogether we think there is cause for concern as anthropogenic impacts are likely limiting individuals from contributing to population growth. We emphasize that our estimate represents a minimum of non-fatal anthropogenic interactions for this population, and we do not know the total number of anthropogenic encounters.

The occurrence of anthropogenic markings on northern bottlenose whales is likely influenced by their curious nature, as they are known to inquisitively approach and follow vessels (Mitchell, 1977), interact with fisheries (Fertl & Leatherwood, 1997; Oyarbide Cuervas-Mons, 2008) and engage in group social behaviour at the surface (Gowans et al., 2001). Other studies have found propeller-vessel strike injuries are common in species that approach vessels and bow-ride (Van Waerebeek et al., 2007) or swim in the wash of the propellers (Visser, 1999). For common bottlenose dolphins in Ecuador, the prevalence of anthropogenic scarring in the population was ~44% (Felix et al., 2018), in the Mayotte archipelago 15% of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) had anthropogenic scars (Kiszka et al., 2008), while 7.5% of false killer whales off Hawaii were found to have anthropogenic scarring (Baird et al., 2014). Entanglement rates have also been found to increase due to particular kinds of cetacean social or foraging behaviour, such as depredation in sperm whales (*Physeter macrocephalus*, Hamer et al., 2012) and open mouth filter feeding in North Atlantic right whales, where 85% of individuals bear entanglement scars (Moore, 2019). While we have observed and are aware of other accounts of northern bottlenose approaching fishing vessels, being hand fed by fishers and depredating trawl and longline fisheries in Newfoundland, Labrador and Baffin Bay (Fisheries and Oceans Canada, 2009; Johnson et al., 2020; Oyarbide Cuervas-Mons, 2008; Wayne Ledwell pers. com.), we are not aware of reports of these behaviours occurring on the Scotian Shelf, or any efforts to document the extent of these behaviours across their range. Additional research in this area would help us understand how the behaviours are spread among individuals, whether they are regionally or demographically isolated, and the prevalence of depredation behaviour within the Scotian Shelf population.

Our classification of probable anthropogenic scars in high quality dorsal photographs limited our analysis to marks that could be recognized based on established literature from other species and expert opinion of entanglement or propeller-vessel strike injuries. This necessarily excluded individuals with unusual scar patterns or large fin notches without associated linear scars. There may be a broader range of possible entanglement injuries for beaked whales involved in offshore fisheries than those recognized from other more commonly observed species. While the trailing edge of dorsal fins can naturally degrade or become tattered over time (Wursig & Jefferson, 1990), entanglements are typically described as scarring on the leading edge of the fin (Azevedo et al., 2009; Baird et al., 2014; Felix et al., 2018; Kügler & Orbach, 2014). Baird et al. (2014) proposed that trailing edge fin scars could potentially occur if whales that became hooked in the mouth thrashed or twisted against the line to break free. Given the low probability of observing beaked whale entanglements, simulation of entanglement mechanics occurring with lines and gear associated with offshore fisheries (e.g. Howle et al., 2018; McLellan et al., 2015) could provide insight on the origin of other unusual scars. Additional analysis of melon and beaks photographs, or aerial imagery of the entire body (e.g. Ramp et al., 2021) would provide another perspective on patterns observed here and comparative estimates of the rate of fisheries interactions.

5.6.5 Conclusion

The contrasting patterns of long-term site fidelity and single sightings, unmarked and distinct individuals, differences between sex-age classes, and observations of anthropogenic scarring found in this study suggest there is still much to be learned about northern bottlenose whale population structure, life history, and threats. While foundational work by Gowans and Whitehead (2001) provided photo-identification methods that have been used for northern bottlenose whales and other species, this study has highlighted that protocols and assumptions about sexing, mark reliability and identifications need to be continuously reviewed to ensure the analysis of larger datasets over longer time periods remains unbiased. Our study found that the prevalence of most mark types is higher for the male-mature male versus female-juvenile sex-age class, which corresponds with patterns found based on molecular sex, but still leaves some uncertainty on whether age or sex is driving these patterns. The increased prevalence of scars could be due to a higher risk tolerance in male-mature males and/or an increase in mark accrual with age. In contrast to our hypothesis on temporal trends, the proportion and rates of most mark types have increased or remained stable rather than decreasing over time. The reasons for increasing trends may be related to an aging population in the Gully. Despite the implementation of the Gully MPA in 2004, northern bottlenose whales face ongoing threats and a risk of injury when they use habitat areas outside the spatial protections provided within the small area of the Gully's Zone one, such as Shortland and Haldimand canyons.

The risk of interactions with vessels and fisheries for northern bottlenose whales has previously been assessed as lower than for inshore whale species, largely due to the reduced density of anthropogenic activities (Brown et al., 2013; Halpern et al., 2008). However, cryptic mortality will bias any estimate of observed anthropogenic injury rate downward, due to low detection rates for whales that do not survive entanglement or vessel strikes (Williams et al., 2011). While we observed some demographic differences in scarring in our dataset, it is also possible that some individuals (e.g., juveniles) suffer higher mortality from anthropogenic interactions and will be excluded from any assessment of scars found on live animals (Byard et al., 2012; Felix et al., 2018; George et al., 2017). Given the uncertainties, we emphasize that this first assessment only tells part of the story, that of non-lethal anthropogenic interactions, which have nonetheless caused a steady number of injuries over the last 30 years. Our estimate indicates the annual rate of injury from anthropogenic interactions is already exceeding the accepted PBR. Combined with new information on the species' slow reproductive rate (Feyrer et al., 2020) and known life history impacts faced by survivors, entanglement and vessel strikes likely present ongoing and significant threats to the recovery of northern bottlenose whales.

5.7 Acknowledgements

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5.8 Authors Contribution Statement

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication. LJF was responsible for project conception, funding acquisition, supervision, data collection, project administration, analysis, data visualization, writing and editing. MS was responsible for data analysis, validation, visualization, writing and editing. JY was responsible for data analysis, validation, visualization, and editing. CS was responsible for data analysis, validation, and editing. HW was responsible for funding acquisition, data collection, project administration, supervision, and editing.

Chapter 6 Evaluating recovery of endangered northern bottlenose whales in a pelagic marine protected area

6.1 Publication Status

Feyrer, L.J., M. Stewart, M.A. MacNeil, H. Whitehead In prep.

This chapter is in preparation for submission.

6.2 Abstract

Globally, there is widespread momentum to implement marine protected areas (MPAs) on the high seas, yet the efficacy of pelagic MPAs is largely unproven. Measuring MPA success can be challenging for long-lived or highly mobile species that characterize pelagic ecosystems. Whales are iconic species and potentially ideal indicators for pelagic MPAs due to their strong patterns of site fidelity that can help define a broad range of spatial protections for important habitat areas. Here we evaluate the efficacy of a pelagic MPA for the Endangered Scotian Shelf population (*Species at Risk Act*) of northern bottlenose whale (NBW) in Canada. Long-term studies on this population demonstrate strong site fidelity to important habitat that includes the highly protected, deep waters of the Gully MPA, established in 2004. However, threats including entanglement, military sonar, and oil and gas activities continue to affect NBW outside the MPA. Using Bayesian and mark-recapture analyses of long-term sightings and photo-identification data to estimate population trends for NBW, we consider the efficacy of the MPA in the light of the change in cumulative human impacts (CHI) of stressors and conservation areas. We found the NBW population declined during 1990-2010, with subsequent recovery beginning after 2004 at a rate of 3-4% per year. The protection of the Gully in 2004 resulted in the largest reduction in CHI magnitude (>2 SD) across NBW habitat. Our study suggests that even small highly protective

pelagic MPAs can contribute to the recovery of cetaceans with targeted placement on areas of strong site fidelity.

6.3 Introduction

To date, the efficacy of pelagic marine protected areas (MPAs) is largely unproven. While theoretical and technical advances have tackled many of the design and enforcement challenges of pelagic MPAs (Allan et al., 2021; Boerder et al., 2019; Game et al., 2009; Geijer & Jones, 2015; Hooker et al., 2011), there is limited evidence that existing MPAs have been successful at meeting their objectives for pelagic predators (Geijer & Jones, 2015; Maxwell et al., 2013; White et al., 2017). Yet there has been an 80% increase in the number of MPAs protecting pelagic areas (> 200m depth) since 2010 (World database on protected areas, 2021, Figure S1.), and area-based conservation goals (i.e., Aichi Biodiversity Target 11, CBD, 2011; Sala et al., 2021) are continuing to drive new international MPA commitments. Thus, the current momentum for MPA implementation seems to exceed efforts dedicated to evaluation, likely due to the significant challenges of monitoring pelagic areas. Establishing whether an MPA is meeting management objectives for representative or vulnerable indicator species relies on measuring population trends at appropriate biologically relevant scales of response (e.g., generation times) (Hooker & Gerber, 2004; Davidson et al., 2012). However, monitoring is logistically difficult and costly, especially for non-commercial species (Game et al., 2009). Further complicating MPA evaluation is that human impacts occurring outside conservation areas and before implementation can overshadow or delay population level responses. To establish whether pelagic MPAs are effective, even for a single indicator, requires evaluating trends within protected areas and across the broader ecosystem at a scale appropriate to the species' life history.

As indicators, cetaceans have become champions for the designation of marine conservation areas around the globe (Hooker & Gerber, 2004; Notarbartolo di Sciara et al., 2016). While MPAs are clearly not always the best tool to address the complex conservation problems of endangered and threatened cetaceans (e.g. Vaquita *Phocoena sinus*, Gerrodette & Rojas−Bracho, 2011; Brownell et al., 2019), area-based protections can be effective at mitigating threats and reducing mortality for cetaceans in areas of national jurisdiction (e.g., exclusive economic zones or EEZ) (Vanderlaan & Taggart, 2009; Notarbartolo di Sciara et al., 2016). However, due to aspects of their life history, including slow reproductive rates with generation times ranging 10-52 years (Taylor et al., 2007), assessing a population response to management interventions requires long-term data. Gormley et al.'s (2012) study of endangered Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand may have been the first to link an increase in survival rates (6%) of a cetacean population to the establishment of an MPA 18 years (or ~ 1.4 generations, Taylor et al., 2007) later. Alongside a reduction in potential fishing gear marks (Wickman et al., 2021), it appears the Banks Peninsula Marine Mammal Sanctuary has been effective in supporting the population's recovery (Gormley et al., 2012).

Despite a lack of quantitative assessments, the growing global momentum to increase area-based MPA targets to 30% by 2030 (World Conservation Congress, 2016) may represent a critical opportunity to achieve significant new pelagic MPAs that could help protect cetaceans. Empirical evaluations of reserve design for cetaceans have provided important insights for management. In 2010, Slooten and Dawson determined that the MPA was likely too small to protect *C. hectori* from future decline, while an assessment of reserves for Boto (or Amazon River dolphin, *Inia geoffrensis*) by Mintzer et al. (2020), identified the long term survival depended on the configuration and protection of core areas of important habitat. Given the risk of creating a new array of target driven "paper parks"(Barnes et al., 2018; Duffus & Dearden, 1993), it is important to evaluate not only whether, but also where and how, pelagic MPAs can improve the quality of outcomes desperately needed by many endangered and threatened cetacean species.

6.3.1 The Status of Northern Bottlenose Whales in Canada

In this paper we consider the recovery and population trends of an Endangered population (Species at Risk Act, (*SARA*) 2002) of northern bottlenose whales (NBW, Hyperoodon ampullatus), located on the edge of the Scotian Shelf off Nova Scotia, Canada between 1988-2019. From the 1880s until the 1970s, the species was heavily whaled across its North Atlantic distribution and, despite being one of the most studied populations of any living beaked whale, their recovery from exploitation has remained uncertain (Whitehead & Hooker, 2012).

Much of what we understand about this population's small size (~143 individuals, O'Brien & Whitehead, 2013) and genetically distinct population structure (Feyrer et al., 2019) is based on 30 years of data collection focused in and around the Gully, the largest offshore submarine canyon in the western North Atlantic. In 2004, the Gully became Canada's first MPA with no-take regulated access restrictions (Horta e Costa et al., 2016; Fisheries and Oceans Canada, 2017). Objectives for the Gully's designation included protecting important habitat for NBW, with six indicators related to NBW population health and recovery identified as indicators for monitoring (Allard et al., 2015; Fisheries and Oceans Canada, 2017)..

The highly protected waters of the Gully's Zone one (Figure 1) is considered the core habitat of NBW in the region, having the highest density of NBW sightings and acoustic detections (Hooker et al., 1999), as well as year-round NBW presence (Stanistreet et al., 2017). The narrow habitat niche of beaked whales is highly dependent on depth, and NBW also occur in lower densities in other areas along the edge of the Scotian Shelf and the Grand Banks in Newfoundland (Gomez et al., 2017; Feyrer et al., 2019). Although some NBW may undertake larger-scale migrations (e.g. 1,000's km),, resident individuals have high site fidelity to the Gully, and nearby Shortland and Haldimand canyons, being observed repeatedly over the entire 30-year period (Feyrer et al., 2021). The concentration of NBW in this area has been attributed to the energetic demands of beaked whales for diving at depth and a dependence on productive areas of high prey density (Hooker et al., 1999; New et al., 2013; Moors-Murphy, 2014; Benoit-Bird et al., 2020).

While human activities are now heavily restricted inside the MPA, across the Scotian Shelf threats with potential demographic consequences for beaked whales continue, including disturbance from oil and gas exploration and development (Lee et al., 2005; Kavanagh et al., 2019), military mid-frequency active sonar (MMFAS) exercises (Simonis et al., 2020), interactions with fisheries such as entanglement and vessel-strikes (Feyrer et al., 2021), and ingestion of plastic debris and contaminants (Hooker et al., 2008, 2019; Lusher et al., 2015; Whitehead & Hooker, 2012). In addition to the Gully MPA, over the last 30 years the implementation of other spatial conservation measures has likely contributed to reducing threats across NBW habitat (Figure 1, Table 1). These include indirect protections related to the moratorium on Atlantic Cod and Haddock trawl fisheries in 1993 (Myers et al., 1997), and direct protections for NBW critical habitat in 2010, which effectively exclude oil and gas exploration. Broad aspatial conservation measures, such as the regulatory listing of the Scotian Shelf population of NBW as Endangered under Canada's *SARA* in 2006, will also have influenced a range of decisions surrounding threat mitigation and permitted activities across the region (Species at Risk Act, 2002). In 2013, seven years after the establishment of the MPA, O'Brien & Whitehead (2013) found the Scotian Shelf NBW population was small but stable. While achieving stability can be considered a success story on a population's path to recovery, for long-lived species, trends observed over timescales less than one generation are unlikely to accurately reflect population level responses to the cumulative impact of human stressors and conservation measures.

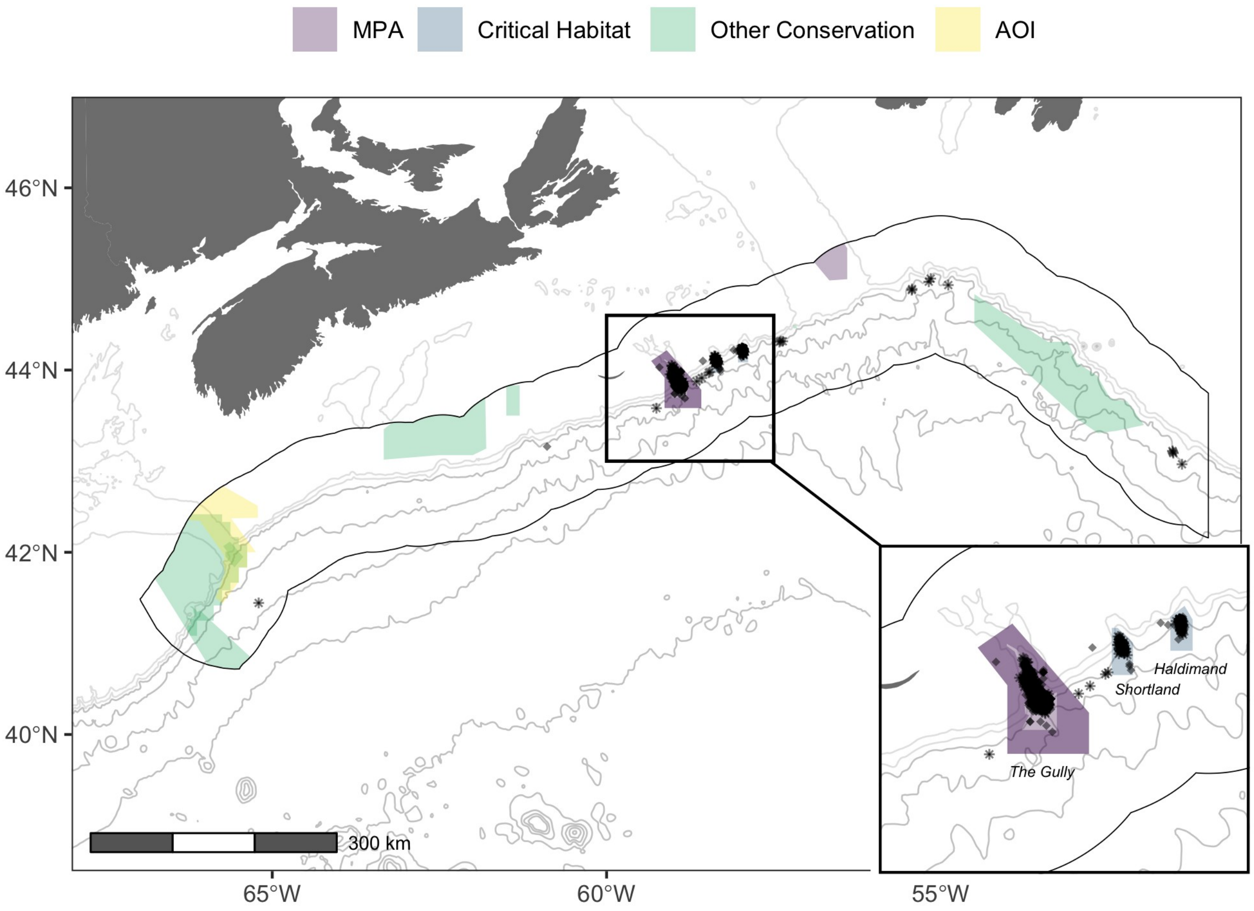


Figure 6.29 Conservation areas with the locations of NBW sightings from surveys 1988-2019 (points) and manually validated acoustic detections from surveys in 2015-2017 (stars). The designated critical habitat areas for NBW include Zone one of the Gully, Shortland and

Haldimand canyons (inset). MPAs are designated under Canada's *Ocean Act*. Other conservation areas include marine refuges and oil and gas exclusions. AOI is the Area Of Interest for a future *Oceans Act* MPA.

Table 6.22 History of marine conservation areas, implementation year, restrictions and approximate size on the outer Scotian Shelf. Size is based on an overlay with the study region and may be smaller than total size of the conservation area. References in footnotes.

Name	Type	First Year	Restrictions	Size km^2
George's Bank Prohibited Area[1]	Other Area	1988	Moratorium on all Oil and gas activities, extended until Dec 31, 2022	9,911
Northeast Channel Coral Conservation Area[2]	Other Area	2002	Restricted fisheries zone and marine refuge ~ 90 percent of the area is closed to all bottom fishing gear including longline, otter trawl, gillnet, and traps	391
Gully MPA Zone one[3]	MPA	2004	Highly restrictive Zone of *Ocean's Act* MPA. No fisheries permitted. O&G not permitted.	477
Gully MPA Zones 2/3[3]	MPA	2004	*Ocean's Act* MPA. Restricted use and access. Hook and line fisheries permitted. O&G not permitted	1887
Lophelia Coral Conservation Area[2]	Other Area	2004	Restricted fisheries zones and marine refuge. Closed a small area (10km^2) around the coral reef to all bottom fisheries	15
Division 3O Coral Protection Zone[4]	Other Area	2007	Restricted fisheries zones and marine refuge. Closed to all bottom contact fishing gear	10,589
NBW Critical Habitat[5]	Species Specific Area	2010	Critical habitat designation covers Shortland and Haldimand Canyons and Zone one of the Gully. Limitation on habitat destruction. O&G unlikely to be permitted. All fisheries allowed	511
Corsair and Georges Canyons Conservation Area[2]	Other Area	2016	Restricted fisheries zones and marine refuge. All bottom contact fishing is restricted with the exception of two small "limited fishing" zones for red crab	2,912
Western Emerald Banks Conservation Area[6]	Other Area	2017	Restricted fisheries zones and marine refuge. Closed to all bottom contact fishing gear	6,588
Laurentian Channel MPA[7]	MPA	2019	*Ocean's Act* MPA. Restricted use and access. Restricted fisheries permitted. Oil and gas and submarine cables not permitted	1,210
Fundian Channel Brown's Bank AOI[8]	MPA proposed	TBD	Area of Interest for Proposed *Ocean's Act* MPA. Proposed restrictions on fisheries unknown. Oil and gas not permitted	5,131

[1] https://www.cnsopb.ns.ca/what-we-do/environmental-protection/special-designated-areas

[2] https://www.dfo-mpo.gc.ca/oceans/ceccsr-cerceef/measures-mesures-eng.html

[3] https://www.dfo-mpo.gc.ca/oceans/mpa-zpm/gully/index-eng.html

[4] https://www.dfo-mpo.gc.ca/oceans/publications/cs-ce/page09-eng.html

[5] https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry/critical-habitat-descriptions/northern-bottlenose-whale-scotian-shelf-statement.html

[6] https://www.dfo-mpo.gc.ca/oceans/oecm-amcepz/refuges/westernemerald-emeraudewestern-eng.html

[7] https://www.dfo-mpo.gc.ca/oceans/mpa-zpm/laurentian-laurentien/index-eng.html
[8] https://www.dfo-mpo.gc.ca/oceans/aoi-si/fundian-fundy-browns-eng.html

6.3.2 Study Objectives

To assess the efficacy of the Gully MPA in protecting the recovery of Scotian Shelf NBW, here we estimate the size of the population and model the cumulative human impact (CHI) of human stressors and conservation areas across their habitat between 1988-2019. Spanning the scale of approximately two NBW generations (15.5 - 17.8 years, Taylor et al., 2007; COSEWIC, 2011), population trends were evaluated using two independent datasets of sightings and photo-identifications of NBW. The extent of threats for NBW over this period was assessed by identifying: (1) spatial patterns of CHI in terms of area, distribution and intensity across their habitat; (2) locations where impacts have improved or declined both inside and outside existing and currently proposed conservation areas; and (3) the area, number and level of protection provided by spatial management measures.

6.4 Methods

6.4.1 Field Methods

Data were collected from 1988-2019 during summer surveys along the edge of the Scotian Shelf from 10-m (1988-1990) or 13-m (1993-2019) auxiliary sailing vessels. During daylight there was a constant watch for cetaceans. Every 3 hours at sea the crew recorded environmental data including: latitude and longitude (Loran-C between 1988-1990; and GPS thereafter), wind speed (Beaufort scale), and estimated visibility (m). All encounters of NBWs sighted were recorded, together with time of sighting, position, and group size. New encounters had to be at least 30-min after the previous recorded encounter. During encounters, photographs were taken of the dorsal fins (both sides) of NBWs, irrespective of the apparent size, sex, or number of markings of each whale. See Feyrer et al. (2021) for detailed methods.

We only used sightings and environmental records that were within Zone one of what is now the Gully MPA, or the designated critical habitat for NBWs in Shortland and

Haldimand canyons (Figure 6.1). During the study years 1988, 1989, and 1990, the studies of NBW were either secondary to those of sperm whales (*Physeter macrocephalus*), or the research emphasis was shared, biasing the sightings per unit of effort measure, so the sightings analysis excludes these years.

6.4.2 Sightings Analysis

We used a condition-corrected number of 3-hr daytime environmental records (06:00-21:00; Atlantic Daylight Time, Z-3) as an index of survey effort. The index of effort in any 3-hr period was the predicted number of sightings in that period from a generalized linear model (Poisson error) regressing the actual number of sightings on wind speed (categorical: Beaufort 0:5, ≥6), visibility (categorical: 0-200m, 201-800m, 801-3,200m, 32,01-12,800m; ≥12,801m), dawn (binary: 06:00) or dusk (binary: 21:00). All environmental data had substantial effects on sighting rates (see Appendix E, Figure E2).

The number of sightings and indices of effort were then aggregated by year (t), month (m: June, July, August; including a few days in early September) and canyon (c: the Gully or other canyon; data were insufficient to separate Shortland and Haldimand canyons). The dependent variable (x) was the number of sightings of NBW during a particular month of a particular year in a particular canyon (e.g., July, 1996, Gully). The data were analyzed in a piecewise-regression multilevel Bayesian framework using map2stan in the rethinking package of R (McElreath 2020; R Core Team, 2019), with a Poisson distribution and varying intercepts (to address overdispersion):

$$x(t,m,c)\quad \mathit{Effort}\cdot e^{\beta_0(t,m,c)+\beta_1\cdot t\cdot(t<T)+\beta_2\cdot t\cdot(t\geq T)+\gamma_1(m)+\gamma_2(c)}$$

$$\beta_{0c}\quad N(\gamma_0,\sigma_0)$$

$$\gamma_0\quad N(-1,1),\sigma_0\quad \mathit{HalfCauchy}(0,1)$$

[allows variation (which is estimated) between sighting rates in different year-month-canyon aggregates]

$$\beta_1,\beta_2,\gamma_1\quad N(0,0.2)$$

[$\beta 1$, $\beta 2$: 95% c.i. for trends in sighting rate of -0.39/yr to +0.39/yr; γ_1: 95% c.i. for proportional differences in sighting rates between months of 0.68 to 1.48]

$$T \quad Uniform[\,]$$

[the year in which the trend in sightings changes]

$$\gamma_2 \quad N(0,1)$$

[95% c.i. for proportional differences in sighting rates between canyons of 0.14 to 7.10]

We fitted models including or excluding all combinations of the year, month, and canyon factors, as well as using a constant linear trend across the study, and fixing the breakpoint at 2004 (the year the Gully MPA was established). Model fit was identified using WAIC (Watanabe-Akaike information criteria; Gelman et al., 2014) score. We also analyzed the data in several other ways (see Appendix E, Table E1, Table E2), but with similar results.

6.4.3 Analysis of Photoidentification Data

Photographic identification methods established for NBW (e.g., Gowans & Whitehead, 2001a) were updated as per Feyrer et al. (2021), and are briefly described here. Photographs of left and right sides of dorsal fins were analysed separately as they could not always be linked to the same individual. Each was given a quality rating (Q) based on the angle, focus, exposure, and visible proportion of fin, with only high-quality photographs ($Q \geq 3$) considered in mark-recapture analysis. All photographs from the Gully, Shortland and Haldimand Canyons were included as individuals frequently move between canyons (Wimmer & Whitehead, 2004). Over the 30-year period the proportion of the population considered reliably marked (those with back indents or fin notches) was 0.48 (SE = 0.013; Feyrer et al., 2021) and estimates were scaled to reflect this.

We fit open mark-recapture mixture models to left and right-side datasets separately, using SOCPROG (Whitehead, 2009). These included parameters for heterogeneity in identification or mortality to allow for different probabilities of identification or survival among individuals (Pledger et al., 2010; O'Brien & Whitehead, 2013; Whitehead & Wimmer, 2005a), mortality, and population trends (linear, piecewise, and a piecewise with a breakpoint set at 2004; the year the Gully MPA was established. Model fit was compared with the Akaike Information Criterion (AIC; Burnham & Anderson, 2002), using the lowest score to select the best model. Confidence intervals for the parameter estimates were calculated from 400 bootstrap replicates. The proportion of reliably marked individuals increased over the course of the study from about 0.44 to 0.53 (Feyrer et al., 2021), so we estimated total population size trends by dividing the number of reliably marked individuals at each year by the proportion of reliably marked individuals in that year (from binomial logistic regression; Feyrer et al., 2021). Aggregated confidence intervals for population estimates were calculated as in Whitehead & Wimmer (2005).

6.4.4 Analysis of Human Impacts

We transformed data on human stressors in the study area into measures of cumulative human impact (CHI) to evaluate where and when the spatial and temporal patterns of human impacts and conservation areas may have contributed to the decline or recovery of NBW on the Scotian Shelf. The study area was defined to encompass the core of NBW habitat using a 50km buffer of the 1000m isobath within the population's range (COSEWIC, 2011) with a raster grid cell resolution of $1000m^2$ (Figure 1, Supplementary methods). The spatial assessment was split into two temporal periods between 1988-2004 (early) and 2005-2019 (contemporary) to assess change over time. The number, area and types of activities restricted by spatial conservation measures was assessed. Change in the distribution and intensity of the six main stressors, including (1) commercial fishing effort; (2) military exercises; development activities, (3) oil and gas exploration; (4) oil and gas development activities; (5) shipping; and (6) sea surface temperature as a proxy for climate change (Table 2) was evaluated. Stressor datasets for each period were normalized, multiplied by a vulnerability weight based on the sensitivity of NBW to each stressor and summed to estimate CHI and compare differences across NBW habitat and over time (sensu Halpern et al., 2015, Table 6.2, Appendix E Supplementary methods). Differences in CHI were assessed in terms of spatial area, intensity and overlap with conservation areas to consider how stressors may be influencing trends in NBW abundance over space and time. Areas of spatial change in CHI, where CHI increased or decreased between periods, were compared where CHI scores that exceeded the root mean square error (RMSE). RMSE, akin to standard deviation, was calculated by square-rooting the mean of the squared differences between the CHI scores of grid cells in the early and contemporary periods, giving an estimate of the average magnitude of the difference between periods (Equation 1, Chai & Draxler, 2014).

Equation 1. Root Mean Square Error

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n} e_i^2}$$

Where: e_i = CHI difference between raster cells in the two periods.

Table 6.23 Human stressors and rationale used for assigning sensitivity weights. Each stressor was evaluated using a 3-point scale based on the frequency, spatial extent and impact of activities on NBW. Weights were applied to normalized intensity values for each stressor and summed by period to assess and visualize cumulative human impacts to NBW across the study area. Appendix E, Supplementary Methods provides additional details on the methods and a description of the spatial data layers used to create the CHI assessment for both periods.

Human Stressor	Frequency	Spatial Extent	Impact	Sensitivity Weight	Data Type	Data Sources
Fishing effort, all gear types combined	Continuous	Broad	Risk for mortality. Threats: entanglement and ship strike known to impact beaked whales.	3	Tons of landings normalized globally by productivity, vessel mins / 1km^2, gear soak time mins/ km^2	1,2
Military practice areas	Intermittent	Broad	Risk of mortality. Threats: Sonar exercises and noise known to impact beaked whales.	1	Modelled based on reported days of activities 2019	3
Oil & Gas operations including wells, pipelines, platforms, production licenses	Continuous	Narrow	Indirect impacts. Threats: anthropogenic noise from surveys, drilling, development and operations; pollution and oil spills.	2	Presence of infrastructure or license	4
Oil & Gas exploration activities including seismic surveys	Intermittent	Narrow	Indirect impacts. Threats: anthropogenic noise from seismic surveys and vessels.	1	Number of surveys and modelled effort for exploration licenses	4
Shipping	Continuous	Narrow	Risk for mortality. Threats: ship strike, noise impacts, contaminants, oil spills.	3	Vessel traffic per grid cell	2
SST Anomalies	Continuous	Broad	Indirect impacts. Threats: distribution range shifts due to warming and unknown resilience to climate change.	1	Number of climatic anomalies relative to baseline	2

[1] (Butler et al., 2019)

[2] (Halpern et al., 2008, 2015)

[3] (Department of National Defense Government of Canada, 2021; Government of Canada, 2021)

[4] (CNLOPB, 2021; CNSOPB, 2021)

6.5 Results

6.5.1 Trends in Sighting Rates

Between 1993-2019 there were a total of 4,563hr of sighting effort, the equivalent of 1,401hr in good conditions (Beaufort 0; visibility >12,800m; neither dawn nor dusk) in Zone one of the Gully MPA and Shortland and Haldimand canyons. The Bayesian models did not support sighting rate differences among calendar months, but estimated that sightings in Shortland and Haldimand canyons occurred at 71% of the rate in Zone one of the Gully MPA (Appendix E, Table E2). The best-supported model estimates a decline in sighting rates of -3.7%/yr (95% c.i. -5.0 to -2.3) until 2010, with an increase of 4.8%/yr (95% c.i. 3.6 to 6.0) thereafter (Figure 6.2).

Systematic trends in group size over the study period would affect the relationship between sighting rate and population size. However, generalized linear models of group size using the Poisson distribution and covariates of month and canyon, found no substantial trend in group size (estimated linear trend +0.0013; SE 0.0013 animals/yr) over the study period.

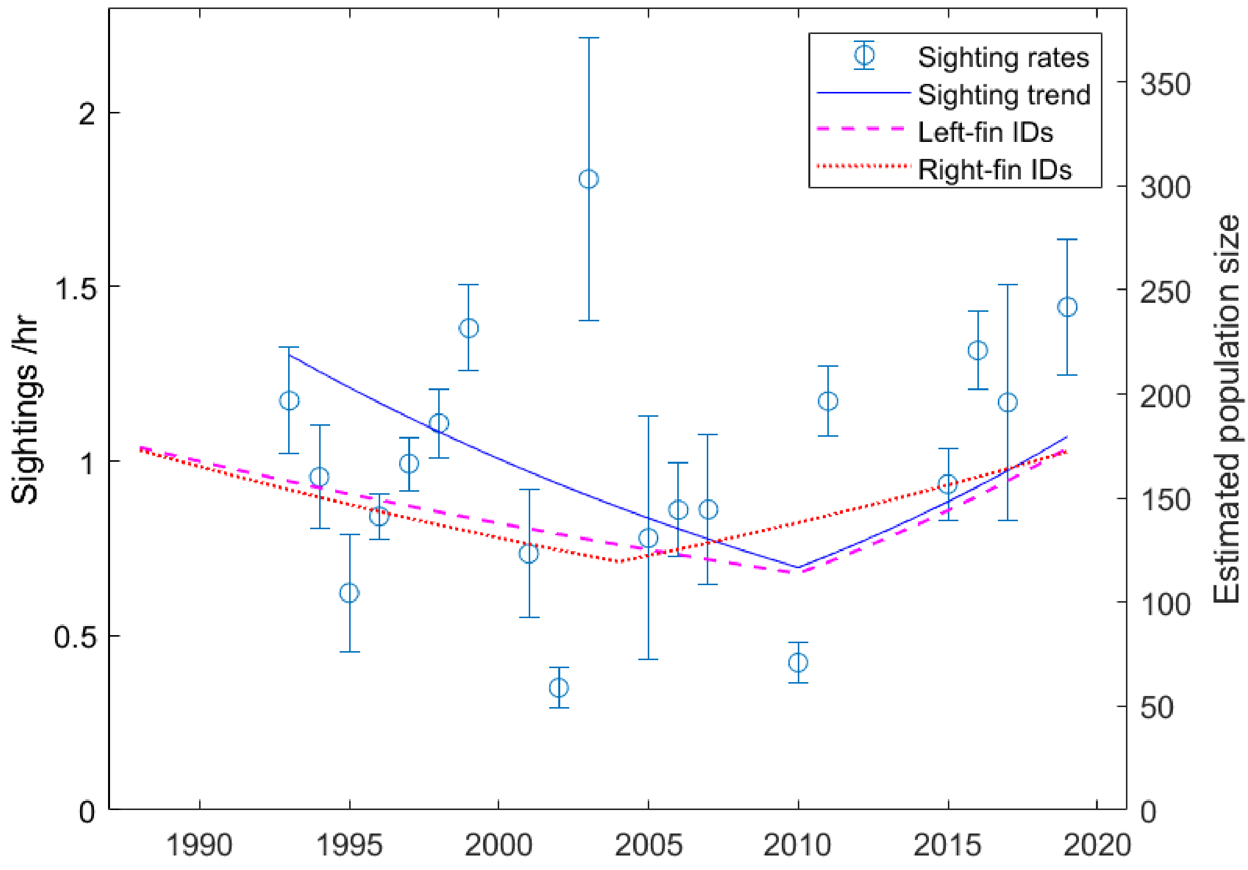


Figure 6.30 Trends in sighting rates (per equivalent hour of perfect sighting conditions) and estimated population trends using mark-recapture models of photo-identifications for northern bottlenose whales in the Gully, Shortland and Haldimand canyons combined. Standard errors for each annual sighting rate were calculated assuming a Poisson distribution. Trend lines for sightings are from a Bayesian variable-intercept model, and for population size from piecewise mixture models incorporating heterogeneity in identification, corrected for changes in the proportion of reliably marked individuals.

6.5.2 Mark-Recapture Population Estimates

There were 211 left (8,075 photographs) and 203 right (6,647 photographs) reliably marked individuals. All models without any form of heterogeneity, and those including heterogeneity in mortality (either with or without heterogeneity in identification), fit substantially worse than the equivalent model with just heterogeneity in identification for both left and right-side data (ΔAIC>8). Thus, only results using models including heterogeneity in identification are presented here (Appendix E, Table E3).

Photoidentification data for the left and right sides of individuals are not entirely independent; as both sides of an animal were likely to be photographed during an encounter, but cannot always be linked, they are analyzed separately. Both left and right-side models, including heterogeneity in identification, had reasonably similar fits (ΔAIC<2.5), with a small preference for a piecewise trend (Appendix E, Table E3). The general piecewise models of left and right sides suggested decreases in population size of -1.4%/year (95% c.i. -5.6% to 0.1% ; left) until 2010 and -1.7% / year (95% c.i -4.,6% to 1.4%; right) until 2004 with an increase of 5.3%/year (95% c.i. 0.8% to 11.1%; left) or 3.0%/year (95% c.i. 0.4% to 7.8%; right) thereafter (Figure 2). At the breakpoint in the piecewise regression (between 2004-2010) the estimated minimum population size for reliably marked individuals was 57.0 (95% c.i. 41.5 to 81.8; left) or 57.7 (95% c.i. 45.8 to 80.1; right). Converted into estimates of total population size at the lowest point in 2004-2010 there were between 113.7 (95% c.i.88.0 to 162.9; left) and 119.5 (95% c.i. 90.7 to 160.7; right) individuals, which by 2019 had increased to 174.0 (95% c.i. 134.2 to 267.4; left) and 172.4 (95% c.i. 116.3 to 238.0; right) (Figure 6.2). The increase in the proportion of reliably-marked individuals over time leads to the estimated trends in total population size being decreased from those in the reliably-marked population by 0.57%/yr (left) and 0.62%/yr (right).

6.5.3 Analysis of Human Impacts

Due to the extent of individual stressors and NBW sensitivity weights, the contribution to CHI in the study area was not equal, with shipping traffic (39%) and fishing effort (37%) representing the proportional majority of CHI intensity scores in both periods (Figure 6.3, Appendix E, Table E4). There was a decrease in the median intensity of oil and gas exploration and SST anomalies, and an increase in the maximum intensity of fishing effort, but the intensity of other individual stressors changed only marginally between the periods of 1988-2004 and 2005-2019 (Figure 6.3, Appendix E, Table E4).

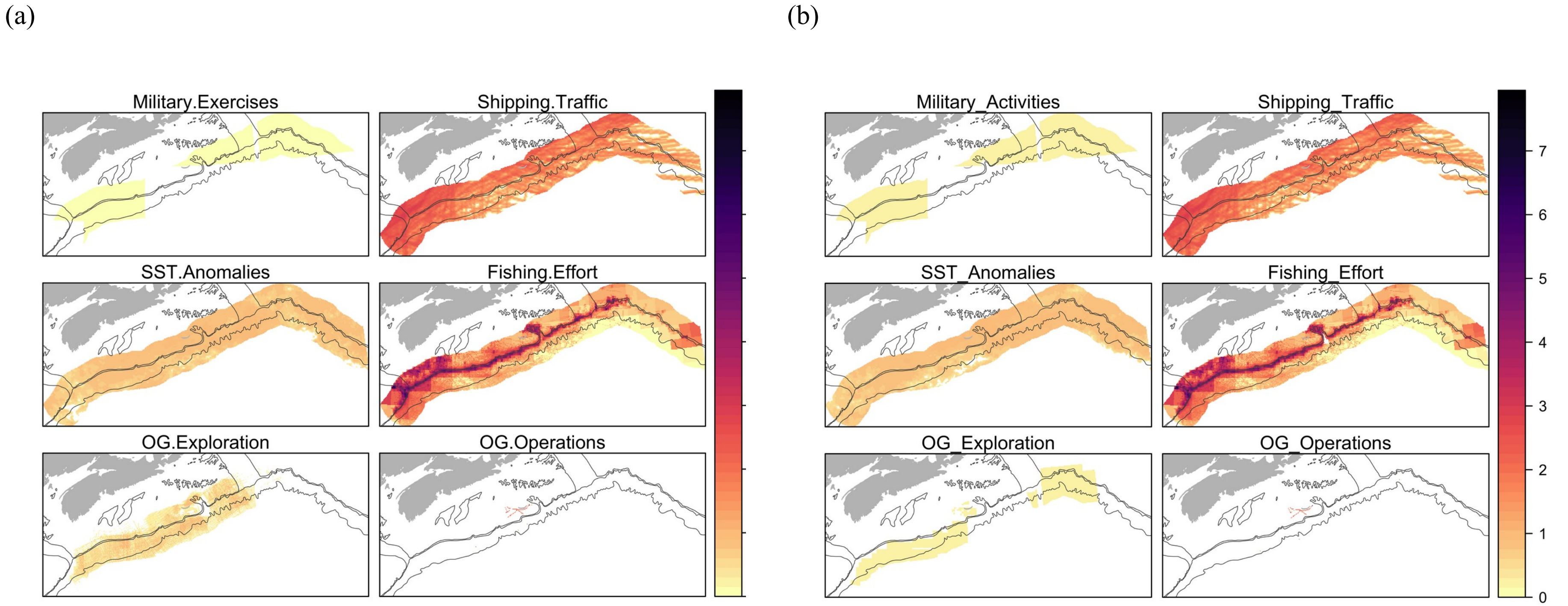


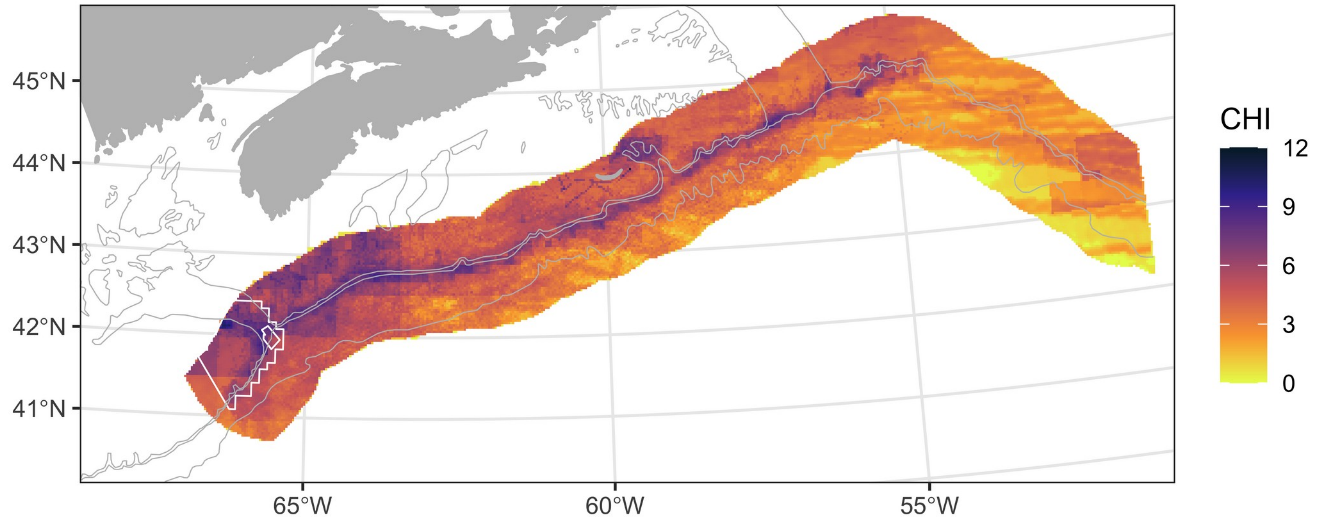
Figure 6.31 Normalized weighted intensity of individual threats to NBW on the Scotian Shelf (a) 1988- 2004 and (b) 2005-2019.

The overall intensity and extent of stressors for NBW estimated by CHI scores, marginally decreased between 1988-2019 (Table 6.3, Figure 6.4), while the number, size (km^2) and activities restricted within conservation areas have increased (Table 6.3, Figure 6.3b, Appendix E, Table E5). Prior to 2004, the median CHI was 3.96, the cumulative sum of CHI scores for the study area was 9.25e+05, the maximum CHI score was 11.2, and conservation areas totalled 10,302 km^2. In the period 2004-2019, the median CHI dropped to 3.75, the cumulative sum of CHI scores decreased to 8.9e+05, the maximum CHI score was 10.6, and the area under spatial protection more than tripled to cover 36,396 km^2.

Table 6.24 Summary of the change in cumulative human impacts (CHI), protected areas and management restrictions between the two periods. The Gully MPA and the restrictive Zone one regulation was enacted in late 2004 and is considered as occurring in the contemporary period. Protected areas with multiple zones are counted as n=1. Contemporary period analysis of conservation areas does not include the Fundian Channel-Browns Bank AOI, which is currently under consideration as an MPA but not yet in effect as of 2019.

Indicator	**1988-2004**	**2005-2019**
Median CHI score	3.96	3.75
Max CHI score	11.2	10.6
Sum CHI score	9.26e+05	8.9e+05
Protected areas km^2	10,302 km^2	36,396 km^2
Protected areas (n)	2	10
Management restrictions	Partial fishing closures O&G exclusions	No take fishing closures Partial fishing closures O&G exclusions
NBW Population trend	Negative, declining	Positive, increasing

(a) 1988-2004



(b) 2005-2019

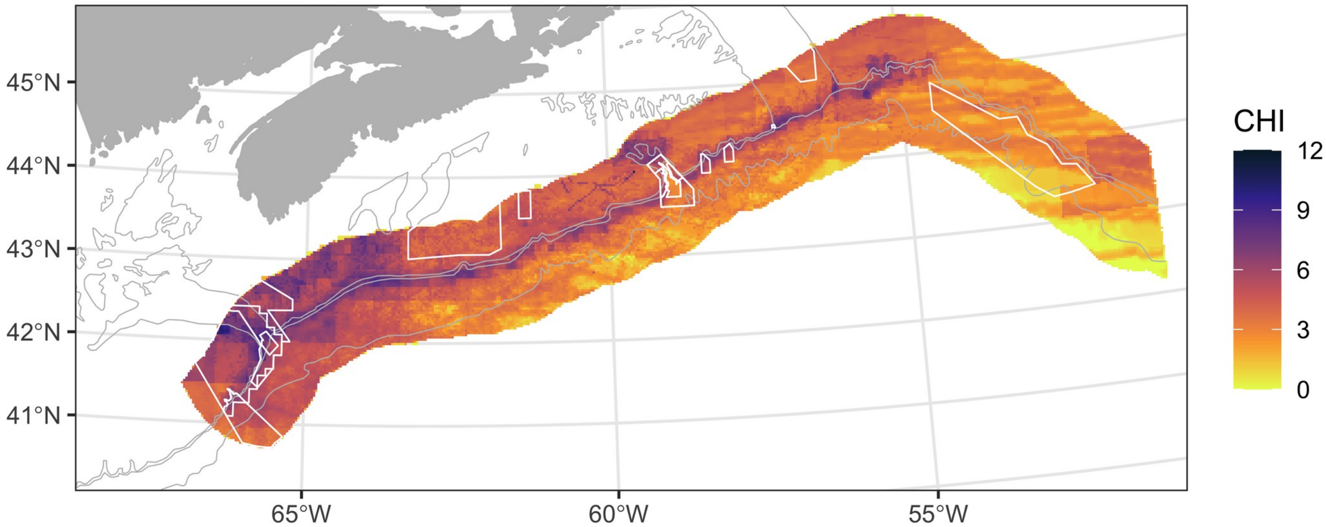
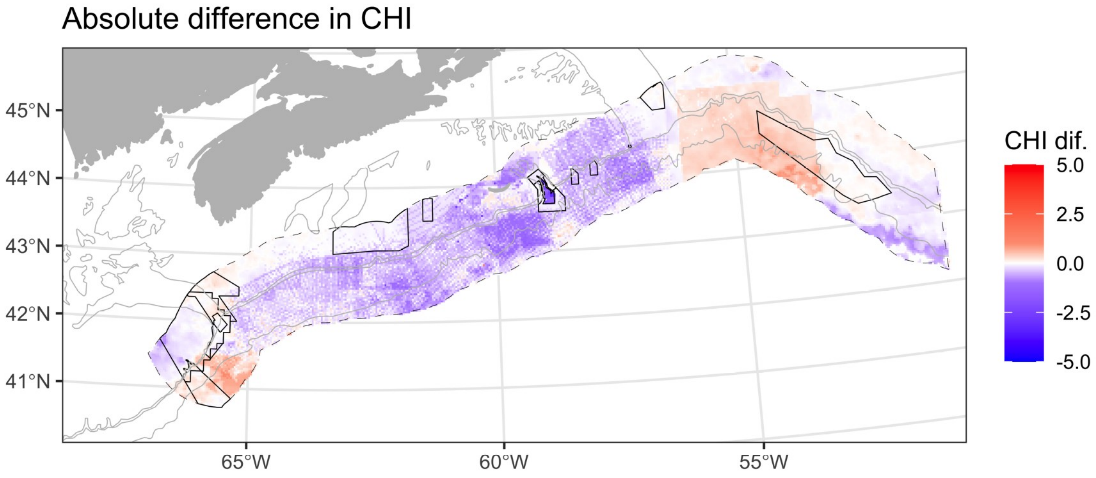


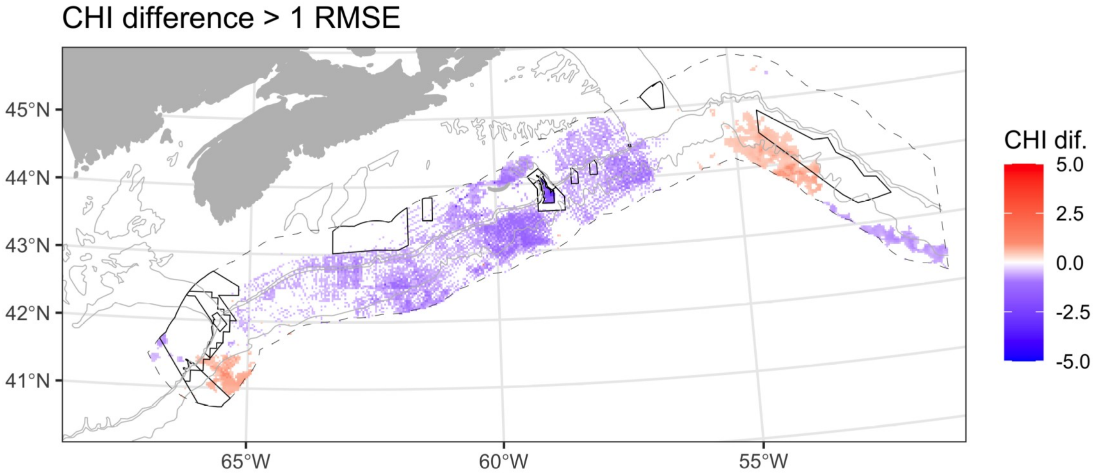
Figure 6.32 Map of cumulative human impacts (CHI) and spatially protected areas in NBW habitat on the Scotian Shelf (a) 1988- 2004 and (b) 2005-2019. Raw intensity data on individual threats (Figure 6.2) was normalized, multiplied by the NBW sensitivity weight (Table 6.2) and summed to understand cumulative human impacts (CHI) to NBW across the study area. Conservation areas in the period are outlined in white (See Figure 6.1, Table 6.1 for details). See Appendix E for detailed methods.

In terms of absolute difference between periods, CHI decreased over a total area of 154,000 km^2, which is approximately double the area of CHI increase (76,300 km^2). RMSE was used as an exceedance threshold to help visualize and identify areas where changes in CHI intensity were above average (Figure 6.5a-c, Appendix E, Table E6). The

RMSE of the difference between CHI intensity in each period was 0.398, and consistent with observations of absolute difference, the areas of decrease in CHI were larger than the areas of increase (Appendix E, Table E6). The greatest decline in CHI, in terms of both area and intensity was seen in the Gully Zone one and nearby deep-water areas to the east and west (Figure 6.5a-c). Areas where above average increase occurred were located south of the Fundian Channel and east along the shelf edge areas of Newfoundland. Significant increases in CHI intensity appear related to increases in SST anomalies and oil and gas exploration activities in Newfoundland in the contemporary period.



(a)



(b)

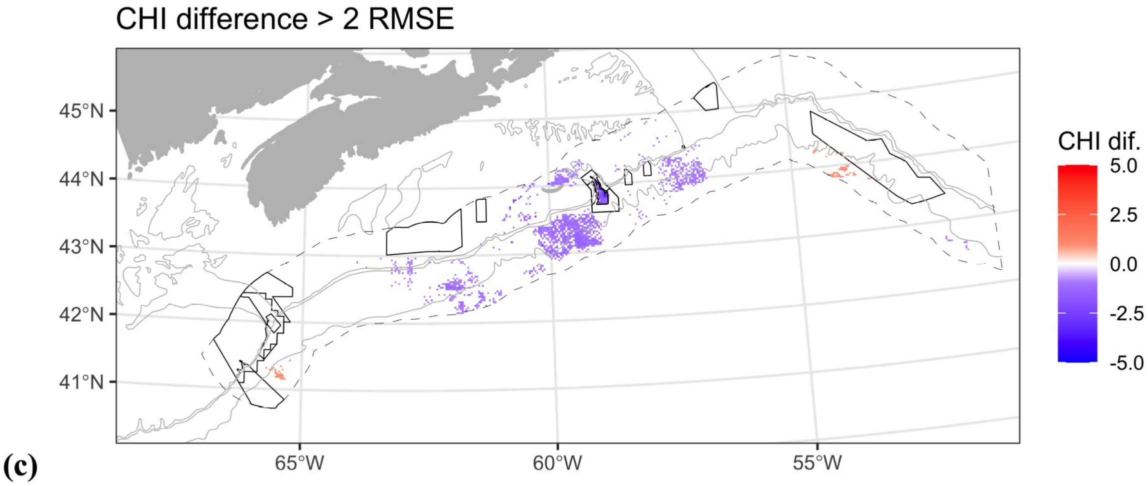


Figure 6.33 Difference in cumulative human impacts (CHI) in NBW habitat on the Scotian Shelf between 1988- 2004 and 2005-2019 as (a) areas of absolute difference in CHI scores, (b) areas where difference in CHI between periods was > 1 RMSE and (c) areas where difference in CHI between periods was > 2 RMSE. Conservation areas in the period are outlined in black.

6.6 Discussion

Population trend analysis on time scales comparable to the long generation times and slow reproductive capacity of cetaceans is rare and difficult. Our perspective on the Scotian Shelf NBW over a 30-year period suggests that the population was declining during 1990-2010, but is now increasing. Negative trends detected in previous analyses over shorter time periods (11 years, Gowans & Whitehead; 13 years, Whitehead & Wimmer, 2005; 23 years, O'Brien & Whitehead, 2013), were not considered significant due to wide confidence intervals and greater support for stable population models. However, the additional years of photo-identification data, a reanalysis of the entire catalogue (Feyrer et al., 2021), and a Bayesian analysis of an independent dataset of sighting rates improved our ability to detect clear population trends.

Changes in population size can occur due to intrinsic or extrinsic factors, and while Canadian NBW populations were targeted by whalers up to the 1970s, this study began tracking the Scotian Shelf population only one generation later. The small remnant population has since been subject to the impacts of ongoing human threats including entanglement and vessel strike from fisheries and shipping (Feyrer et al., 2021; Chapter 5), pollution and contaminants from oil exploration and development (Hooker et al., 2008), and noise disturbance associated with all activities across the area (Whitehead & Hooker, 2012). The legacy of whaling also poses ongoing impacts; low genetic diversity (Feyrer et al., 2019; Chapter 3) and the potential for inbreeding, compounded with a slow reproductive rate (Feyrer et al., 2020; Chapter 4) will have increased the vulnerability of the population to other stochastic demographic and environmental risks (Shaffer, 1981; May, 2019), further inhibiting recovery post whaling. That the decline reversed beginning in the mid-2000s suggests that threats diminished, genetic-reproductive limitations were overcome and/ or stochastic events were favourable. While genetic diversity or life history is unlikely to change over the timescales considered here, extrinsic environmental stressors such as the impact of human activities in important areas of NBW habitat have changed. However, the history of human activities on the Scotian Shelf bioregion (Department of Fisheries and Oceans, 2005) not only predates this study period, but includes many temporally coincident and spatially overlapping threats to NBW, making it difficult to retrospectively identify a single trigger associated with observed population trends. Thus, we built a model of cumulative human impacts to visualize and understand how changes in the spatial-temporal patterns of intensity in stressors and conservation actions over this period may have played a role in NBW recovery.

Between 1988 and 2019, eleven conservation areas were implemented in the study area, with only two areas existing before the 2004 implementation of the Gully MPA. It seems that Canada’s commitment to international MPA targets have resulted in a marked increase in the area and number of spatial conservation measures in the contemporary period (Fisheries and Oceans Canada, 2019). While not all conservation areas identified here meet the criteria as highly protected MPAs that can be counted towards Canada’s targets (Horta e Costa et al., 2016), at a minimum they either restrict some fishing activities (e.g., primarily bottom contact fisheries) or exclude oil and gas exploration. Our estimate suggests the increase in Scotian Shelf NBW began sometime shortly after the Gully MPA was implemented in 2004. However, with long-lived mobile species like

NBW, trends will reflect a response to conservation efforts and human impacts across broader temporal and spatial scales.

The mobility of large marine predators has often brought into question the effectiveness of spatially static MPAs for protecting their populations (Hooker et al., 2011). Largely due to the requirements for intensive monitoring over periods matching the scale of response, only a few studies have been able to demonstrate that long-lived cetaceans can benefit from coastal or near shore MPAs (e.g., Gormley et al., 2012), but there are no studies that we are aware of that have made the same link for pelagic MPAs and cetaceans. Our retrospective evaluation found the recovery trend for NBW roughly coincident with the timing of the protection of the Gully in 2004, an event that resulted in the largest reduction in CHI magnitude across NBW habitat. If even small pelagic MPAs can contribute to the protection of cetaceans, the focus of further evaluation should shift to how we can make pelagic MPAs more effective. Not all MPAs are created equal and the success of the Gully MPA for NBW is likely due to the targeted placement on important NBW habitat and no-take exclusions on a range of human activities in Zone one. In addition, while NBW are still vulnerable to threats outside Zone one of the Gully MPA, the adjacent zones of the MPA may buffer against spill over impacts and the proximity of other conservation areas can mitigate against increases in overall CHI. While pelagic cetaceans may never be easy to monitor as “good indicators” of MPA success, as flagship and umbrella species, consideration for the effective conservation of whale populations provides strong incentives and support for a high standard of MPA design and regulatory protection (Hooker & Gerber, 2004; Hooker et al., 2011; Horta e Costa et al., 2016).

Our additive model of CHI was largely a heuristic exercise to scope the distribution and intensity of changes in human activities and conservation areas in the Scotian Shelf region. Maps of CHI focus on stressors for NBW, but provide useful visualizations of human activities that are also concerns for the conservation of other whales and pelagic species (e.g. Sowerby’s beaked whales (*Mesoplodon bidens*), North Atlantic right whales (*Eubalaena glacialis*) and leatherback sea turtles (*Dermochelys coriacea*). Excluding commercial fisheries and military exercises from Zone one of the MPA contributed to significant reductions in the core area of NBW sightings, despite small overall differences in CHI across the study area (Figure 6.1). With the exception of Newfoundland's Division 3O Coral Protection Zone, which is a partial fisheries closure, all significant increases in CHI (> 1 RMSE) occurred outside of conservation areas and seem related to increases in oil and gas exploration and SST anomalies. These results suggest that higher levels of protection within conservation areas would help mitigate impacts that are less predictable and unregulated (e.g., SST anomalies) as well as buffer against those impacts that are difficult to quantify and model spatially as they can travel beyond their source (e.g., noise, contaminants, or ghost gear). Conservation areas with comprehensive restrictions on allowable human activities, such as Zone one of the Gully MPA, can be effective in reducing impacts on species and ecosystems not only within their own borders but across a broader area.

Detailed studies of cumulative effects in the environment have found that due to interactions or synergistic relationships between stressors, the intensity of patterns can result in multiplicative or exponential impacts, relationships that our CHI model is too simplistic to reflect. However, without more detailed data on stressors and effects pathways, and an analysis of ecological relationships (e.g., a network scale cumulative effects assessment, Beauchesne et al., 2020) our assessment likely underestimates CHI. The limitations of individual stressor data may also affect the assessment of CHI, as nearly 80% of the relative intensity of CHI was due to the combination of fishing effort and shipping traffic. This proportion reflects not only the extensive area occupied by these activities, but also reflects the sensitivity weight assigned to address concerns for the potential impacts of entanglement and vessel strikes (Feyrer et al., 2021). While we had the temporal data to enable comparisons for most layers, the areas southeast of Newfoundland were not completely covered by longline effort or seismic survey datasets. In contrast, we had full spatial coverage of shipping intensity but had to use the same layers in both periods to visualize the distribution of shipping intensity across the region. Finally, to account for the impact of sonar exercises (MMFAS) on NBW, intensity data were modelled across the grid of predefined military areas, as detailed data on these exercises is unlikely to ever be made public due to national security concerns. The currently available layers serve the intended purpose of our CHI analysis; however, we recognize that increasing the coverage or resolution of underlying data layers may paint a more comprehensive picture and potentially highlight areas where the impacts of individual stressors were underestimated. While additional refinements may reduce some uncertainties regarding individual stressors, we think they are unlikely to change the overall broad-scale temporal and spatial patterns in CHI.

Our understanding and interpretation of CHI and NBW population response to human stressors was challenged by the larger uncertainties associated with the impacts of climate change on marine species and ecosystems. The impact of our changing climate has been linked to shifts in the distribution and range of cetacean species from around the world (e.g., bowhead whales (*Balaena mysticetus*), Chambault et al., 2018). Such alterations have also highlighted the ways climate change can exacerbate or interact with other stressors, such as those faced by North Atlantic right whales off eastern Canada, and challenge the implementation of spatial conservation measures designed to protect previous areas of important habitat (Meyer-Gutbrod et al., 2018; Record et al., 2019). NBW are assessed as having moderately high vulnerability to climate change based on traits including site fidelity and prey specificity (Albouy et al., 2020). Increasing ocean temperatures and ocean acidification are predicted to contract the range of NBW northwards under future climate scenarios (Lambert et al., 2014). Recognizing that climate change poses a potentially significant and increasing stressor, we include differences in SST anomalies based on Halpern et al.'s (2015) modelling and rationale. However, our interpretation of specific areas of concern is limited by a poor understanding of how and when temperature extremes trigger ecosystem shifts, and the scale of response between trophic energy transfers in surface waters and bathypelagic ecosystems, where NBW feed. While our knowledge of the effect pathway is imperfect, over the period of this study, SST anomalies have increased in the region and were included as an indicator of overall ecosystem change.

This retrospective evaluation of patterns associated with NBW population recovery provides a much-needed success story for marine species and conservation. Our analysis of CHI suggests that reducing stressors through spatial conservation areas has supported the recovery of NBW. It appears even small conservation areas can be effective if restrictive measures mitigate against the most impactful stressors and target areas of important habitat. Of course, the interplay of internal and external factors contributes to the growth or decline of small populations, providing highly variable responses over shorter time scales. Detecting population trends in long-lived endangered species, like cetaceans, involves interpreting monitoring data on a scale relevant to species' reproductive potential. As such we caution that as population recovery is an ongoing process, the temporal stability of this trend is uncertain. The Scotian Shelf NBW are still vulnerable to stochastic and demographic events, given their small population size, and would likely benefit from additional restrictions on stressors currently permitted within other habitat areas. In terms of the efficacy of pelagic MPAs, it seems that protecting the integrity of important habitat is likely more important than the size of conservation areas. Despite the uncertainties, we are optimistic that the protections in place today will continue to improve outcomes for future generations of NBW.

6.7 Acknowledgements

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recognize that Dalhousie University continues to benefit from its location on Mi’kma’ki, the ancestral and unceded territory of the Mi’kmaq people.

Chapter 7 Discussion

7.1 Homage to the Gully or Why Are There So Many Kinds of Beaked Whales?

In my study of northern bottlenose whales, I used multiple methods to investigate the evolutionary and ecological patterns and processes structuring their populations. I considered scales of connectivity, ranging behaviour, gene flow, demographic threats and life history contributing to the species contemporary populations and abundance in Canada. As long-lived whales adapted to the ecosystems and habitat at the southern edge of their species' range, members of the Scotian Shelf population should be considered a unique evolutionary lineage, containing diversity important to the future resilience of NBW. The extreme variation found across and within species of beaked whales and the high diversity of species found in the Gully, echoes the kōan posed in Hutchinson's (1959) seminal review – Why are there so many kinds of animals? Though beaked whales are bigger than beetles, the wide array of subtly segregated sympatric ziphiid species leads the curious to question the origin of patterns and processes driving their interspecific differences and reproductive isolation. While efforts to conserve evolutionarily significant diversity are at the forefront, Hutchinson's provocative musings on the limitations of productivity, niche requirements and the "mosaic nature of the environment" advanced in 'Homage to Santa Rosalia' suggest that the answers are at their core ecological. The value of understanding large mammal biodiversity wasn't lost on Hutchinson either, as he pointed out the "immense scientific importance" of studying the ecology of the large mammals of Africa, particularly while it was still possible under natural conditions, as the results could support "the establishment of greater reservations and National Parks than at present exist." Reflecting on Hutchinson's call to the practical necessity of fieldwork, I remain optimistic that despite the threats, research on such "wonderful animals" as beaked whales remains critical to the protection of their populations and ecosystems.

My thesis builds on the hard earned data, analysis and insights into beaked whales gleaned by others who have over the last 30 years re-examined museum collections and fossils dragged from the bottom of the sea, developed new methods and technology that can survive the record breaking dive depths of ziphiids, and stubbornly persisted with the challenges of long-term field studies. They have done much to advance our understanding of beaked whales, as an area of research previously considered opportunistic or impossible for most species, has become a field active with revisions to Ziphiidae's phylogeny based on an expanding list of extinct and extant species (Bianucci et al., 2016; Dalebout et al., 2014; Kawatani & Kohno, 2021; Yamada et al., 2019), vulnerability to threats (e.g., Military Sonar; Cox et al., 2006; Miller et al., 2015; Simonis et al., 2020; Wensveen et al., 2019), and the accumulation of a relative wealth of empirical data on species abundance, distribution, population structure, life history, behaviour and social organization. In a family of enigmatic tusked oddities, NBW have also distinguished themselves from other beaked whales not with their teeth, but by their social nature and curiosity towards *us*. In a family typically characterized as avoidant or skittish, NBW's interest in approaching vessels nearly led to their downfall in the whaling era. The relative accessibility of NBW has done much to facilitate research, broaden understanding and generate interest in the biology and conservation of beaked whales.

It might be said that extraordinary creatures require extraordinary habitat. Supporting a rich community of cetacean species diversity, including at least four species of beaked whales, the Gully exists among a patchy mosaic of submarine canyons in the western North Atlantic (Moors-Murphy, 2014; Stanistreet et al., 2017). Formed by inter-glacial melt waters 150-450 kya, the Gully was likely still terrestrial habitat when the radiation of ziphiid species alive today came to be. Imagining the Gully's marine transformation serves as a reminder that Earth's history of glaciation and changing climate have imposed strong selective and vicariant forces. In the marine environment, notorious for its lack of obvious boundaries, large physical habitat structures like the Gully are rare, and provide stable boundaries relative to other oceanographic features. Such discrete productive areas in the ocean are ecologically attractive sources of habitat, nutrients, aggregations of prey, and mating opportunities (Block et al., 2011; Hyrenbach et al., 2000; Moors-Murphy, 2014). Over evolutionary time physical habitat can persist where currents or productive fronts may not, occasionally creating refugia, preserving or diverging character traits and supporting the multiple niche dimensions of a diversity of species that may otherwise only be found in the fossil record.

A day in the Gully without the spouts, snouts and persistent hungry foraging clicks of a population of NBW would be hard to imagine; however, their presence there is not a given. Whaling clearly had both genetic and demographic effects across the range of NBW (Feyrer et al., 2019; Whitehead & Hooker, 2012), leaving a small declining Scotian Shelf population to contend with the cumulative impacts of other largely unmitigated industrial activities in their habitat. Because the energetic budget of NBW survival is tight (Benoit-Bird et al., 2020; New et al., 2013), their reproductive capacity is limited (Feyrer et al., 2020; Taylor et al., 2007), constraining their abundance, distribution and recovery to deep water areas with high concentrations of prey biomass, such as the Gully. Without reductions in the overall cumulative impact and restrictions against fishing activities in zone one of the MPA, we may not have seen the trend of their decline change course. Records of high site fidelity spanning decades (Feyrer et al., 2021) and NBW foraging recorded year round in the Gully, Shortland and Haldimand canyons (Moors, 2012; Stanistreet et al., 2017), suggests the productive ecosystems of the Gully (Strain & Yeats, 2005) and nearby canyons offer a reliable supply of prey and few reasons to leave (Feyrer et al., 2021; O'Brien & Whitehead, 2013; Wimmer & Whitehead, 2004).

Reinforced by the availability and stability of high-quality prey resources, site fidelity of NBW to the Gully may also be influenced by other features of habitat. Discrete features such as canyons or seamounts are by definition discontinuous, although proximity and scale play a role in the frequency of interchange between such structures. While NBW appear to move regularly between the Gully and adjacent canyons (scales of 50km) with residency in areas over periods of weeks, the Scotian Shelf is separated from the Arctic Ocean by the island of Newfoundland and the shallow waters of the Grand Banks (scales of 3000km). While unpublished tagging studies of NBW indicate some animals may travel longer distances (e.g., between the Davis Strait and the Flemish Cap; S. Ferguson, pers. com), we know little about the ontogeny of individuals who made these trips, who they might have been travelling with or why. There is also a contrast in oceanographic conditions across the NBW range. The cold Labrador current, which dominates the waters of NBW habitat in the Canadian north, is only one small part of a confluence of currents found around the Gully, including the waters exiting the Gulf of St. Lawrence, occasional intrusions of warm core-rings from the Gulf Stream, and the salty North Atlantic (Strain & Yeats, 2005). Across a similar area, Stanley et al. (2018) found a climatic gradient followed a cline in the genetic structure of five marine species with a phylogeographic break point occurred in the vicinity of the Gully, indicating evidence of macroecological drivers of cryptic population structure. Given the restricted high latitude distribution of their genus, preferences and adaptations to fine scale environmental differences across the range of NBW habitat likely still play a role in structuring their population dynamics.

In addition to reducing connectivity by decreasing the overall density of NBW across the North Atlantic, whaling may have contributed to the fragmentation of populations in other ways. The largest recorded NBW killed by whaling was an 11.16m male (MacLeod, 2006), over two meters larger than the maximum size of male NBWs reported today by Ellis & Mead (2017). As whaling is known to have targeted older larger individuals, the impacts of size selection would have potential consequences for dispersal life history and social learning (Whitehead, 2010, 2017). Left in the wake of the whaling era, the size of smaller remnant populations and a loss of cultural knowledge, would leave few risk takers available to explore potential frontier habitat outside the known areas of the Gully and northern Labrador. Fast forward a few generations, and despite the lack of records of NBW taken by whalers from the waters off Newfoundland, I had regular encounters with groups of NBW in my field work around the Sackville Spur on the northeast corner of the Grand Banks in 2016 and 2017, habitat that has since been confirmed through passive acoustic monitoring as a year-round foraging area (Delarue et al., 2018). There were no photo-identification matches or resights of NBW seen on the Sackville Spur between other areas. Combined with the distribution of NBW from acoustic surveys around the edge of the Grand Banks (Figure 1.2) with the apparent increase in strandings around Newfoundland over the last 20 years (Table A1) and low but subtle genomic structure indicating Newfoundland NBW contain diversity unique from other areas (de Greef et al., 2021), suggests this region of important habitat may represent an area of mixing or range expansion for NBW populations.

Unlike other species of cetaceans with a long oral history of interactions and relationships with indigenous peoples (e.g., gray whales (*Eschrictius robustus*) Béland et al., 2018; but see history of NBW use dating back to the 16th century in the Faroe Islands in Bloch et al., 1996 and medieval Iceland (Szabo & Frasier, 2020)), we know almost nothing about NBW population dynamics or ecology prior to commercial whaling. Despite technical advances, the era of post whaling cetacean research is young compared to the period of exploitation and the life expectancy of many cetaceans. Genetic reconstructions of demographic history have provided useful insights on trends in NBW abundance and expansion over evolutionary time, as well as recent bottlenecks, likely due to whaling (Feyrer et al., 2019). Contemporary analysis of ecological relationships (e.g., Hooker et al., 2002) and potential cultural distinctions (e.g., Eguiguren et al., 2021) are limited to data collected over the last 30 years. A challenge common to understanding and managing previously exploited species, is that measurements of their contemporary abundance and distribution likely reflect “shifting baselines” (Pauly, 1995). As this generation of NBW recover from whaling, their population’s growth, range and evolutionary structure are occurring within altered ecosystems, where the ocean temperature, prey distributions and threats reflect a different set of conditions than those faced by their ancestors. Current observations of NBW distribution, population size and habitat use may represent temporary or unstable trends, rather than carrying capacity, preference or suitability. Analyses and interpretations need to further consider how NBW may be navigating the present seascape of human threats. Beaked whales and NBW are challenges in this regard due their strong behavioural responses to human activities, particularly military sonar (Miller et al., 2015; Wensveen et al., 2019), boat avoidance in other ziphiids and vessel attraction and fisheries depredation by NBW (Johnson et al., 2020; Oyarbide Cuervas-Mons, 2008). Long-term datasets have proven to be critical resources for finding such signals in the noise, as have methods to measure cumulative impacts (e.g., Halpern et al., 2015).

We may never know the specific pathway of effects or have the long-term baseline to assess population recovery or other changes at the "right" spatio-temporal scale. However, we do know that cetaceans with high site fidelity, long life spans, restricted ranges and prey specializations, like beaked whales, will be inherently limited in their ability to respond to altered ecosystems. Despite the uncertainties, the vulnerability of NBW to ongoing and unprecedented climate change needs to be recognized. In future climate scenarios the range of NBW has been predicted to contract northwards (Lambert et al., 2014), leaving whales in the Gully, at the southern limit of their current range, with a choice to stay and make do, or leave and try their luck elsewhere. Changing conditions across the North Atlantic will have some yet to be determined influence on the Gully's ecosystem, and although the MPA appears to have reduced cumulative human impacts for Scotian Shelf NBW over the last decade, whales will often move to other areas in response to prey availability (e.g., as in the case of right whales, Meyer-Gutbrod et al., 2018; Record et al., 2019). Whether it is possible to mitigate the threats of climate change for cetaceans is also uncertain, given our poor understanding of the natural and potentially changing dynamics of the benthic-pelagic realm, the issues and needs of NBW are difficult to anticipate, however their vulnerable status cannot wait for an answer (Woo-Durand et al., 2020). Proactively working to support and maintain NBW recovery may not directly address the questions of climate change, but in reducing threats where and when we can for NBW, we make space for their future resilience. However, climate change is not just a future problem, it is actively underway, and as species contract or shift their range away from the "warm edges," climate-related local extinctions and extirpations have already become widespread across marine species (Wiens, 2016). Despite finding an trend of increasing NBW abundance over the last decade, intrinsic and external threats, including climate change, leave the small Scotian Shelf population vulnerable to local extirpation or extinction. While the MPA contributed to reducing impacts in their critical habitat over the last decade, we cannot take for granted that NBW will always be able to find shelter in the Gully.

As specialized foragers with high energetic demands, where would NBW go instead of the Gully? Moving east, towards the cooler waters of the Labrador current, there are a series of other submarine canyons along the slope edge. However, the highest concentration observed across the scattered distribution of NBW acoustic detections, appeared along the western side of the Sackville Spur, which is an undersea sediment drift (Maillet et al., 2005). It is unclear whether and how habitat features in between the Gully and the Sackville Spur sustain stable or ephemeral concentrations of prey, and whether there may be ecological differences between the diets of NBW in these areas. While NBW stranded in Newfoundland regularly have stomach's full of squid beaks (Ledwell et al., 2020), they are also known to engage in depredation behaviour of ground fisheries off the Grand Banks (Oyarbide Cuervas-Mons, 2008), and in the Davis Strait (Johnson et al., 2020). The status and population structure of NBW in northern areas also remains uncertain. Ongoing genomic analyses (de Greef et al., 2021) suggests low levels of population structure in NBW across the North Atlantic reflect a latitudinal cline. Though our interpretation of gene function in NBW is limited to paralogues in other species, genes under selection and associated with temperature regulation appear to correspond with a clinal pattern. More research is required to understand the function of these genes and how they may reflect adaptations of the Scotian Shelf population to the warmer waters of lower latitudes or constrain their use of other habitat environments.

My thesis made a significant contribution to our appreciation of NBW in the waters around Newfoundland, finding evidence of their scattered distribution along the slopes of the Grand Banks, and an area of concentrated sightings indicating important foraging habitat on the Sackville Spur. Although there were no photo-identification matches between areas or years, whales genetically sampled in 2016 and 2017 shared a mixed population structure suggesting connectivity with the Scotian Shelf, Davis Strait and potentially other populations. Given a poor understanding of NBW's historical distribution around Newfoundland from whaling records, it remains to be determined how new observations and limited genetic analyses should inform their delineation as management units. The current designated unit (DU) boundary between the Scotian Shelf and the northern population (Figure 7.1), is arbitrarily drawn across the middle of the Grand Banks, reflecting the political barrier of Canada's exclusive economic zone (EEZ), rather than an evolutionary or ecological distinction or a habitat discontinuity. That the shelf edge extends beyond the EEZ, means the Sackville Spur is in an Area Beyond National Jurisdiction (i.e., ABNJ) and poses another question for the management of NBW in Newfoundland and Canada (Marotte, 2017). While the Canadian Newfoundland Offshore Petroleum Board (CNLOPB) continues to promote "100 new wells by 2030" (Government of Newfoundland and Labrador, 2019), permitting exploration and exploitation of oil and gas in the hinterland of the Flemish Cap (Wangersky, 2019), and North Atlantic Fisheries Organization (NAFO) permits international fishing fleets to drag and trawl the slope of the Sackville Spur up to the line of Canada's EEZ, there are few policies or legal tools empowering the assessment and protection of wildlife populations that extend into international waters (Marotte, 2017). My thesis identified and established areas of occurrence off Newfoundland, however assessing their status requires more data, effort and political will.

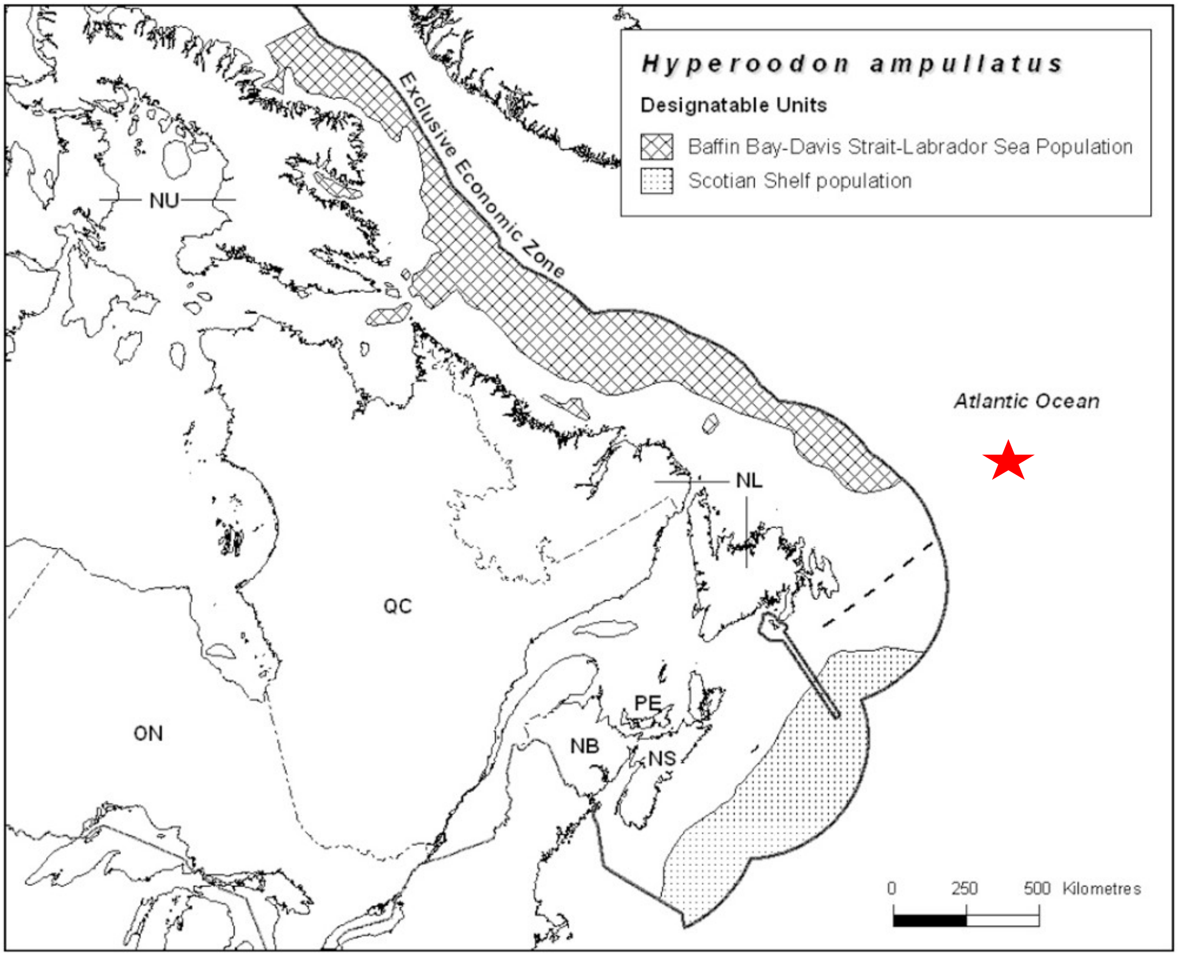

Figure 7.*34* The distribution and range of NBW in Canada with the current COSEWIC designated unit boundaries identified. The broken line represents the arbitrary boundary between the two recognized DUs. It follows the boundary separating Division 3L from Divisions 3N and 3O used by NAFO. The Sackville Spur concentration is indicated with a red star. Map modified from COSEWIC (2011).

Protecting NBW in the international waters off Newfoundland represents a conundrum faced by many Canadian populations of cetaceans and migratory species occupying the High Seas (Rochette et al., 2014). Unfortunately, the nature of ongoing and expanding oil and gas development in the area surrounding the Sackville Spur may have irreversible effects before additional research or effective spatial management tools can be put in place. As an important habitat area midway between the Scotian Shelf and Baffin-Bay-Labrador Davis Strait DUs, threats to the Sackville Spur may represent a sink for population dynamics as NBW expand and recover. Precautionary management enshrined in Canada's commitments to the CBD, *SARA* and the Oceans Strategy already provide a mandate to consider threats to Canadian species in light of data deficiencies. The resilience and recovery of NBW on the Scotian Shelf and in Canadian waters will depend on continued population growth, maintaining adaptive genetic diversity and protecting connectivity between important habitat areas, including those just outside the EEZ.

7.2 Significant Themes

My thesis contributed significant new knowledge to our appreciation of NBW and beaked whales. Our limited understanding of the patterns of population structure in beaked whale populations, demonstrate a mixture of panmictic and parapatric processes across a range of spatial scales; however, the diversity of species within their family, indicate there is still more to learn. For NBW, my genetic studies indicate there are at least two subpopulations within Canada. I also discovered the location of a consistent foraging area off the Sackville Spur in Newfoundland, which may represent an area of mixing and expansion but reflects a previously undescribed important habitat area. I found evidence in stable isotopes from archival specimens that NBW provide prolonged maternal care, which has implications for their energetic budgets and lifetime reproductive potential. Through the reanalysis of 30 years of photographs from the Scotian Shelf (Stewart, 2018; Yeung, 2019), I built the first fully digital photo-identification catalogue for NBW as well as the first catalogues for Newfoundland and the Davis Strait, necessary for calculating population estimates using mark-recapture methods (Feyrer et al., 2021). In my review, I identified a trend in reliable mark rates in the Scotian Shelf population that needed to be accounted for in my estimate of population size as well as a consistent pattern of anthropogenic scars. Combined annual gain rates of entanglement or vessel strike marks were higher than potential biological removal indicating that these threats have been underestimated for NBW. Long-term trends for the Scotian Shelf population indicate that they have recently recovered from a decline, corresponding to a reduction in cumulative human impacts across their habitat in the period following the protection of the Gully MPA. This work provides the first evaluation of change in cumulative stressors indicating that a pelagic MPA can help protect cetaceans.

There are three themes that emerge when taking a broader view of my thesis research. The first is that while beaked whales are specialists, their study and conservation requires a generalist approach. I am not the first to take on the research challenges of NBW, but as I have demonstrated, there was no one technique or method that would have been sufficient to get the answers I sought. I learned through my collaborators and mentors the necessity and complexity of integrating diverse datasets and analytical methods to address the research challenges associated with elusive data poor species, like beaked whales.

The second theme is the implications of my work for the management of NBW and beaked whales. While many of my objectives and questions were outlined in the NBW recovery strategy as knowledge gaps, the answers I found are not all easily applied. The population unit appropriate for the management of NBW and other beaked whales may elude the hard boundaries as well as the definitional confines of the COSEWIC Designated Unit due to the challenges of small sample sizes, low density and levels of migration, subtle structure, shifting baselines, cryptic threats, and recovering populations. The term 'recovery' appears to be management shorthand for reducing mortality and monitoring, however reproduction is the only way a small population can increase in size, outgrow their vulnerability to stochastic risks and improve their status (Lacy, 2000; Shaffer, 1981). While there is little a manager can do about beaked whale reproduction, understanding reproductive potential is critical for modelling populations and estimating the impacts of mortality they can more easily control. Learning that NBW, one of the most well understood species of beaked whales, have a poorly understood history of fisheries interactions highlighted the risks of relying on traditional observer reporting to determine the extent of cryptic threats. My work also evaluated whether management interventions such as MPAs can still make significant contributions to the mitigation of overall threats. NBW's long-term site fidelity for the Gully, and the impacts of disturbance to the energetics and life history of beaked whales (Benoit-Bird et al., 2020; Czapanskiy et al., 2021), makes a strong case that protecting important habitat areas for ziphiids can be effective (Hooker et al., 2002, 2011).

Finally, the third theme is the utility and necessity of field science and long-term empirical data sets for the science based management of beaked whales. Though not without its challenges, some form of direct observation is essential to creating and validating (or falsifying) our evolving understanding of the world of beaked whales with data. Fieldwork constrains our theories, informs our research questions, and reminds us that knowledge does not just drop out of a text book or a computer model, it must be acquired at some point through the experiential part of the scientific method. Gathering empirical data on beaked whales is an achievement, but it is largely a long game. Data collection in the Gully began over 30 years ago, surviving the whims of funders, risk-averse university administrators and growing data management challenges. Each three-week trip offshore contributed to what would become the most important long-term field study on NBW and eventually, Sowerby's beaked whales (*Mesoplodon bidens*) (Clarke et al., 2019; Hooker et al., 2019; Whitehead, 2013). Initially opportunistic, the NBW research program grew into a dedicated multi-year study with standardized protocols that *collected the data*, which identified the vulnerable size of the Scotian Shelf population and their dependence on the Gully (Gowans et al., 2000; Hooker et al., 2002; O'Brien & Whitehead, 2013; Whitehead et al., 1997; Wimmer & Whitehead, 2004). The scientific weight behind the message that NBW were at risk was clearly influential for their conservation, and would be used to advocate and justify the protection of the population and their critical habitat (COSEWIC, 2011; Fisheries and Oceans Canada, 2017). The legal recognition of the Scotian Shelf population as Endangered reinforced the significance of the field study, providing the mandate and the funding for ongoing research on NBW. My thesis would not have been as interesting, useful or possible without the legacy of field work on NBW and other beaked whales conducted by previous graduate students and researchers, or the interest and support of managers dedicated to their conservation. Fieldwork on NBW continues to be necessary to address outstanding questions on the fundamental biology of beaked whales, understand long-term variation and monitor the status and recovery of their populations across the North Atlantic.

7.3 Summary of Thesis Chapters

In the following section I summarise each of my research chapters and identify how they contributed to the aim of my thesis, which was to advance our understanding of NBW population structure, life history and threats to improve the scientific management and conservation of beaked whales in Canada and around the world.

In chapter two I addressed the question of population structure for cetaceans through a systematic literature review and meta-analysis of trends with particular consideration for odontocetes. Due to their cryptic nature and challenges associated with offshore data collection there were only a few studies (N = 6) that examined sub-specific population structure in beaked whales. Studies suggested that differentiation can occur at a range of scales, across ocean basins for Gray's beaked whales (*Mesoplodon grayi*; Thompson et al., 2016) to sub-ocean areas for Cuvier's beaked whales (*Ziphius cavirostris;* Dalebout, 2005) and Baird's beaked whales (*Berardius bairdii;* Kitamura et al., 2013), and smaller regional areas for NBWs (Dalebout et al., 2006; Feyrer et al., 2019). Despite few barriers to dispersal, there are clearly ecological and evolutionary processes driving the diversity found within the currently defined twenty-three species in the beaked whale family. Consideration for the potential of undefined population structure in beaked whales is an important challenge for their conservation and management, as evolutionarily significant units may contain critical adaptive diversity necessary for their future resilience in the light of ongoing climate and ecosystem level changes (Lambert et al., 2014; Perrin, 1991; Sgrò et al., 2011).

In chapter three I evaluated the evidence for evolutionary population structure and historical demography within NBW using high resolution genetic markers, giving particular consideration to the distinction of the Scotian Shelf and individuals sampled in a newly identified habitat area off Newfoundland. Scotian Shelf NBW share a number of distinct haplotypes and were distinguished as a unique population with microsatellites but were not monophyletic. Due to sample size and low overall diversity, the genetic affinity of NBW off Newfoundland was uncertain, suggesting an area of mixing with no clear population distinction. While my research confirmed the genetic distinction of the Scotian Shelf DU from other samples, continuous acoustic detections but discontinuous genetic sampling across the region cannot resolve a distinct boundary with other putative unit(s). Across cetaceans, NBW were found to have nearly the lowest genetic diversity (Louis et al., 2020), which can be symptomatic of rapid demographic declines as a consequence of exploitation or historical ecosystem changes. Demographic reconstruction found differences in the signal of expansion after the last glacial maximum, with the Scotian Shelf population undergoing a steep decline in genetic diversity, at a temporal scale coincident with increasing human activity in the North Atlantic. Despite few barriers to movement in the marine environment, the case of NBW demonstrates that beaked whale species can exhibit fine scale population structure but may require a relatively large number of samples to detect.

In chapter four I used stable isotopes to infer dietary changes and identify the weaning age of NBW from annual growth layers in a large archival collection of teeth. Ironically, this study would not have been possible or perhaps necessary if NBW had not been the target of commercial exploitation. Consideration for a "sustainable catch", led whaling era scientists to measure and record all aspects of NBW that were killed, preserving a *memento mori dente* as part of their efforts (Benjaminsen & Christensen, 1979). Evidence for prolonged maternal investment challenges previous estimates of the inter-calf interval and reproductive potential for NBW and merits the revaluation of assumptions made for other species of beaked whales. The limited number of tooth specimens available in natural history collections, combined with the destructive nature of sampling and high individual variability (Feyrer et al., 2020) make it unlikely that a study of this scale could easily be replicated for other species of beaked whales. However, this work contributes to the growing number of studies highlighting the value of collections, new analytical methods and the preservation of specimens of opportunity to increase our understanding of the fundamental biology for data poor beaked whale species (Gol'din, 2014; Lockyer & Garrigue, 2021; Smith et al., 2021).

In chapter five I conducted a longitudinal study of the origin and stability of marks found over thirty years in a photo identification catalogue of Scotian Shelf NBW. Highlighting the importance of the Gully for NBW and the significance of site fidelity in beaked whales, was the proportion of individuals with a sighting history spanning 10 years or more. An increase in reliable marks over this period, may indicate an aging population and should be a consideration in mark recapture studies. The results published in Feyrer et al. (2021) were significant in demonstrating that NBW interactions with fisheries and vessels occur more frequently and likely pose a higher risk for animal welfare and survival than previously thought based on historical observations and bycatch records. The broader implications for beaked whales and small cetaceans are that bycatch records are not representative of rates of entanglement and that more dedicated effort is required to understand the mechanics of interactions with offshore fisheries and mitigate their impacts to beaked whales.

In chapter six I consider long-term NBW population trends in light of an assessment of change in cumulative human impacts, including conservation efforts across their habitat. The growing global momentum to protect 30% of the world's oceans has presented a critical opportunity to protect important habitat, but the efficacy of Marine Protected Areas (MPAs) for pelagic species, including whales is largely unproven (Allan et al., 2021). My evaluation of change in cumulative human impacts considers what threats were mitigated by MPAs and found that the highly restrictive no-take area of the Gully's Zone one was effective in significantly reducing overall threats for NBW. Following a decline in NBW abundance in the first half of our study, the period after the implementation of the Gully MPA has seen an overall increase in population size. This is a good news story for NBW and offers hope for the conservation of other beaked whales and pelagic species, as it indicates that even small highly protective pelagic MPAs can contribute to the recovery of cetaceans with targeted placement on areas of high site fidelity.

7.4 Future Research Opportunities

What does the next 50 years of beaked whale research look like? As outlined in Hooker et al. (2019), it likely looks like more of the same: continuing long-term data collection, developing new methods and techniques for monitoring and measuring their populations, revisiting assumptions about species and life history requirements, and advancing the tools necessary for effective mitigation of human impacts. While we race to catch up with the current roster of research, at some point in the future we can expect the discovery of new species of beaked whales to slow. A broad general understanding of the beaked whale family has been built up from a series of exploratory investigations of individual species, with occasional comparative studies across the better known Ziphiid species (e.g. Dalebout et al., 2008; MacLeod, 1998, 2006; Macleod et al., 2003). Relying on accumulating inferences drawn from "model" taxa, (e.g., NBW) may not help us understand other beaked whale species if we do not know what makes them fundamentally different in the first place. Why are there so many species of beaked whales, is as compelling a question now, as it was 60 years ago. Advancing our progress towards the answer(s) requires additional theoretical consideration for the ecological and evolutionary patterns and processes driving the structure of their populations. Phylogenetic studies have highlighted how patterns of radiation may reflect convergent evolution (Bianucci et al., 2016), niche exploitation (Macleod et al., 2003) and sexual selection (Dalebout et al., 2008), however the similarities in body size, subtle teuthivorous niches and habitats shared among ziphiids suggests the ecological separation may be more behavioural. Does the diversity of ziphiid species and populations matter? If we want to make good on our commitments to preserving biodiversity, I argue yes. It may never be possible (or practical) to collect sufficient data to understand all the variation within ziphiid species. While data are still required, synthesizing what we do know of the phylogeny, life history traits, ecology and behaviour of beaked whales into a theoretical framework could help us understand where and whether inter-species inference is useful. As human industry continues to extend further offshore, understanding the impacts of human activities on ziphiids has emerged as a top research priority. Here again, theoretical work on diversity could contribute to our appreciation of potential differences in the vulnerability of beaked whales. The knowledge gaps are so numerous for some ziphiids and the field is so ripe with research questions that it may not yet be mature enough for such a unifying framework. However, a more theoretical approach could also help identify strategic research priorities with broader impact and interpret the implications of studies across species. A body of knowledge is more than the sum of its parts, and understanding the members of Ziphidae in terms of their subspecific population structure, ecology, behaviour, response to human impacts and changing environmental conditions, will require synthesizing a comparative understanding where and when we can.

Throughout my thesis chapters I have identified a number of research gaps specific to NBW. I conclude my discussion with a brief overview of areas for future research related to NBW conservation and management.

Small peripheral populations are expected to be less resilient to demographic removals and further reductions in genetic diversity due to the lack of connectivity and increased risk of inbreeding. Despite a number of genetic resampling events, due to low variation across genetic markers I was unable to assess evidence for inbreeding within the Scotian Shelf population. Monitoring NBW using low depth genomes or higher resolution markers on an ongoing basis could help understand whether inbreeding may be an issue limiting the recovery of the Scotian Shelf DU. Distinguishing whether NBW in Newfoundland represent the expanding edge of northern populations, a range shift or another distinct sub-population unit requires additional analysis of tissue collected from strandings, NBW on the Sackville Spur as well as other whales found in the intermediary areas between the population centers.

Extended weaning in NBW suggests long-term maternal relationships are important aspects of their ontogeny, survival and require further study. Beyond meeting nutritional needs, maternal relationships are a primary source of social learning, cultural transmission, and site fidelity. However, previous analysis found preferred associations in NBW were strongest between mature males, similar to the social organization of common bottlenose dolphins (*Tursiops truncatus*) (Gowans et al., 2001; O'Brien, 2013). Addressing questions on NBW social structure, mating systems and dispersal will have important implications for understanding other drivers of population structure and mechanisms of inbreeding avoidance relevant to their conservation (Parreira & Chikhi, 2015).

Beaked whale research has relied heavily on analysis of specimens of opportunity, and as other studies have demonstrated (e.g., Smith et al., 2021) further stable isotope analysis of historical tissue data from whaling, museums and strandings could provide additional insight on ecological population structure in NBW over broader spatial scales. Given uncertain providence and uneven distribution of archival tissues, simulation modelling of NBW spatial behaviour across the region of interest (e.g., Carpenter-Kling et al., 2019; Trueman et al., 2019) can be used to generate hypotheses, estimate the power of sample design and predict the range of potential ecological gradients relevant for understanding patterns and processes of ecological population structure.

Photo-identification is an important tool in any long-term study of beaked whales, and as digital catalogues grow in size, they can become challenges for data management. Little is known about the site fidelity of NBW outside the Scotian Shelf, and outside of relatively invasive methods like satellite tagging there are few other methods for measuring short term movement patterns. Potentially, evolving methods for the acoustic identification of individuals or populations (e.g., Eguiguren et al., 2021) will allow passive acoustic monitoring to provide a wealth of new monitoring information on movements and other dynamics in future. Although there were no matches between the Scotian Shelf and smaller datasets collected in Newfoundland and the Arctic, photo-identification efforts in Iceland have been ongoing over the last decade. Image analysis employing artificial intelligence (AI) has already been applied to match individuals within the photo-identification catalogues of other species of cetaceans (e.g., *Delphinidae* spp., humpback whales (*Megaptera novaeangliae*), right whales (*Eubalaena glacialis*); Weideman et al, 2017; Khan et al., 2020) and likely will prove to be an important tool for testing error rates in hand matching and integrating catalogues of NBW developed for other areas.

Incidents of NBW entanglement have been documented in trawl, longline and trap fisheries (Feyrer et al., 2021), however the mechanics of entanglement in the various offshore gear types and related injuries and animal welfare concerns are poorly understood for NBW. Whether the issue is related to under reporting by fisheries or the mechanics of NBW breaking free and avoiding detection could be addressed through automated video observation systems on board fishing vessels, which is currently not required for Scotian Shelf fisheries. Experimental studies of line and hook break strength and injuries (e.g., McLellan et al., 2015), simulation modelling of incidents (Howle et al., 2019), and spatial analysis of fisheries effort across NBW habitat areas (e.g., Hines, et al., 2020) would help characterize and identify solutions to mitigate the risks associated with different types of gear or fisheries.

In characterizing the threats to NBW across the Scotian Shelf, there were a number of uncertainties. We should be able to refine our understanding of temporal or spatial risks with additional data. Oil and gas activities were a challenge given the inconsistencies in what was available from the Nova Scotia and Newfoundland regulatory authorities, but also due to embargoes on more recent surveys and updated websites that allow map viewing but not spatial data downloads preventing further independent analysis. Similarly, without historical data on military activities, I had to model the general distribution of risk posed by military sonar based on contemporary reports of activity days. The calculation of cumulative human impacts could not have occurred without the Halpern et al. (2015) dataset, however their global resolution data was coarse compared to spatial impacts associated with these activities (e.g. shipping). Increasing the resolution and extent of underlying data on stressors and NBW habitat areas (e.g., through species distribution models) could help improve our understanding of the impacts of threats to beaked whales occurring across their range and identify targeted areas where MPAs may be most effective.

7.5 Conclusion

NBW are a top marine predator in the Gully and have been present across the pelagic ecosystems of the North Atlantic since the Miocene. They have evolved specialized adaptations to exploit a deep water teuthivorous niche, tolerating pressure, lack of oxygen and various degrees of cold. Surviving multiple glacial epochs, NBW have, like many large mammals suffered large population declines during the Anthropocene. One of the most well-known species of socially gregarious beaked whales, there are still a number of critical areas for future research and conservation concern. Although I discovered a new area of important habitat off Newfoundland and identified a positive trend in population size for the Scotian Shelf NBW, over the scale of NBW life history and evolution, these are recent developments. Whether future generations of NBW remain resilient to ongoing threats inside and outside the Gully, including a rapidly changing ocean, likely depends, at least in part, on the seriousness of our commitment to the conservation of biodiversity when and where we find it.

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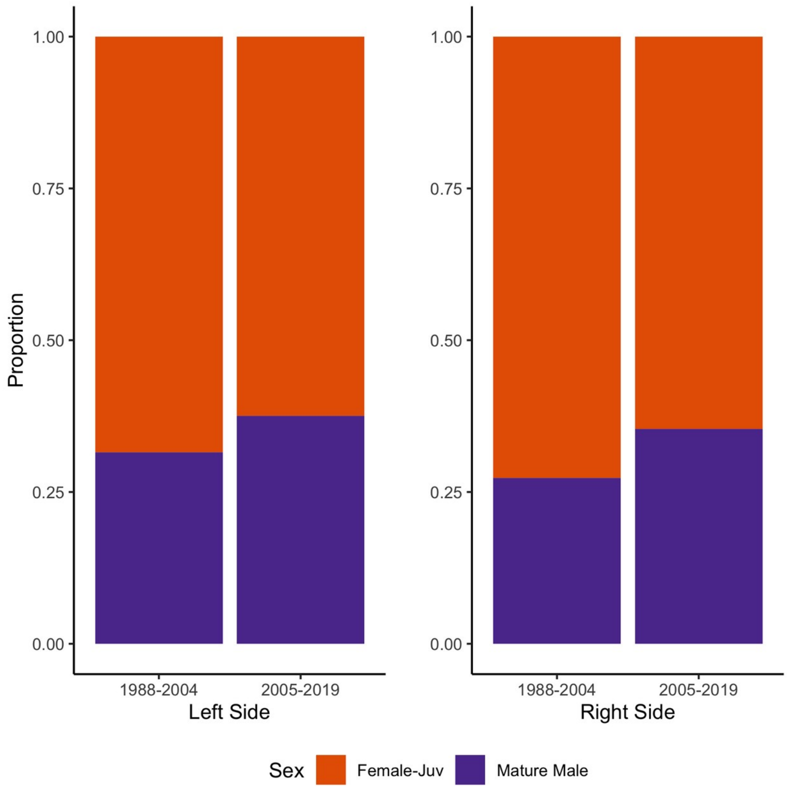
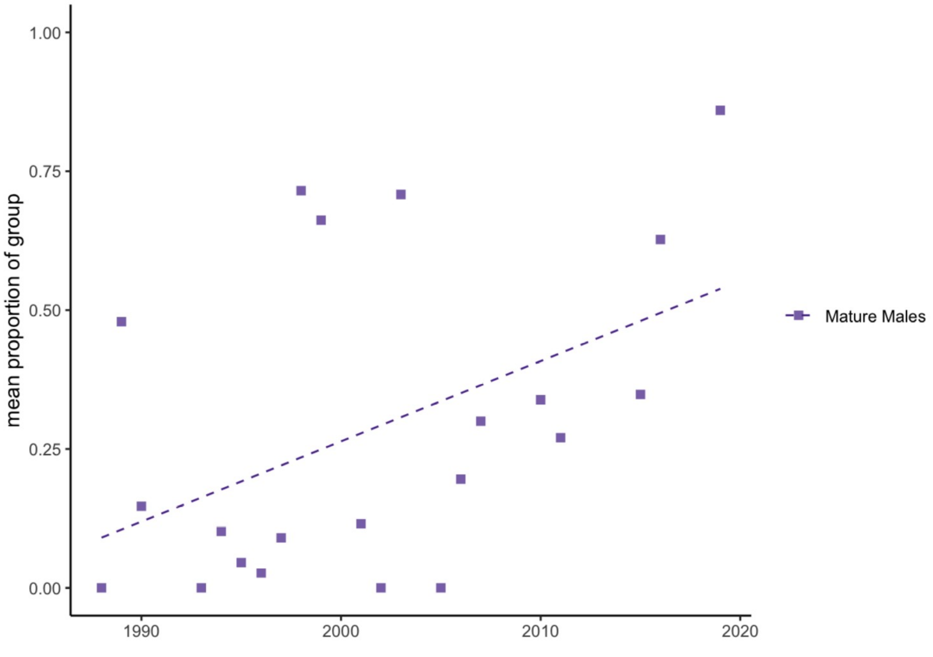
APPENDIX A Chapter 1

Table A1. Records of NBW strandings and entanglements in Newfoundland. Records provided by Whale Release and Strandings Network of Newfoundland, DFO Newfoundland and Sergeant & Fisher (1957)

Year	Date	Incident Type	Sex	Age	Location
1953	27-Jul	Whaled	Male	Adult	Dildo Arm, Trinity Bay
1990	05-Sep	Entangled in squid trap, released	Unknown	Adult	Dildo Arm, Trinity Bay
2002	08-Mar	Unconfirmed reported stranding	Unknown	Unknown	Makovik, Labrador
2004	22-Jul	Floating dead	Unknown	Unknown	Fortune Bay
2004	10-Aug	Live stranding, died	Female	Adult (10)	Culls Harbour, Bonnavista
2004	10-Aug	Live stranding, died	Male	Juvenile (3)	Culls Harbour, Bonnavista
2005	03-Aug	Live stranding, died	Female	Juvenile	Milltown, Bay d'Espoir
2007	19-Mar	Beached dead	Male	Unknown	Golden Bay, Cape St. Mary's
2007	26-Jun	Beached dead	Unknown	Adult	Lawn, Burin Peninsula
2008	26-Sep	Stranded	Unknown	Unknown	Hopedale, Labrador
2011	07-Jun	Floating dead	Male	Adult	West of Lamaline
2011	26-Jun	Beached dead	Unknown	Unknown	Mattis Pt.
2014	16-Jun	Beached dead	Unknown	Unknown	Stephenville Crossing
2019	12-Aug	Live stranding, died	Female	Adult	Harbour Mille, Fortune Bay
2021	16-Feb	Live stranding, died	Female	Adult	Boyd's Cove
2021	07-Mar	Beached dead	Male	Adult	Musgrave Harbour


Proportion
1.00
0.75
0.50
0.25
0.00
1988-2004
2005-2019
Left Side
Right Side
Sex
Female-Juv
Mature Male

(a)


mean proportion of group
1.00
0.75
0.50
0.25
0.00
1990
2000
2010
2020
Mature Males

(b)

Figure A1. The proportion of Mature Males observed on the Scotian Shelf between 1988-2019 (a) from photo-identification analyses of melons FJ (orange) and MMM (purple) and (b) as a proportion of groups from sightings records. L. Feyrer unpublished data.

APPENDIX B Chapter 2

S1. Methods for Systematic Review and Data Extraction

A systematic literature review was conducted using a keyword search of the Web of Science and Biological Abstracts databases for all peer-reviewed English publications that explored population structure below the species-sub-species level across all families of Cetacea. 1,831 records were returned using the keywords: "*population structure" OR "stock" OR "sub-population" OR "evolutionary significant unit" OR "ecologically significant unit" AND "whale" OR "dolphin" OR "cetacean" OR "odontocete" OR "mysticete" AND NOT "shark*"*. All references were imported into the systematic review management software Coevidence, which detected and removed 345 duplicate references. I then screened the title and abstract of the remaining 1,486 articles for studies specific to cetacean population structure below the species/sub-species level. Of these studies, 510 were considered potentially relevant and subjected to a full text review for inclusion based on eligibility criteria (Table B1).

Table B1. Eligibility criteria for full text screening review

Exclusion Criteria	Inclusion Criteria
Study did not actually assess population structure or distinctiveness	Study must be focused on assessing the population structure below the species-subspecies level
Strictly about historic population structure (except as a line of evidence for current population structure)	Study must be of a wild cetacean population
Strictly about management of populations	
Strictly about social structure	
Studies of population size or range estimates	
Studies attempting to assign extralimital observations, strandings or unknown specimens to known populations	
Studies discussing population structure assessment methods without an application, e.g. primer development, statistical models	
Studies with the goal of differentiating sub-species differences	
Articles that assessed the population structure of multiple species in one paper	

Studies without location information

For all 356 studies that met inclusion criteria, reference information including Author, Title, Abstract, Journal and year of publication was exported into Microsoft Excel for data extraction. The variables and coding schema were determined based on the research questions, issues for population structure research raised by authors through a review of key literature and a feasibility assessment of an initial subset of studies. Variables were either directly extracted from the study (such as N values), coded during extraction (presence/ absence), or extracted as raw text for review and coding post extraction (e.g. Study goals), as described below. A more detailed extraction of 40 variables (see Table B1) was limited to studies of odontocetes, providing an in-depth overview of experimental design, cetacean population structure and conservation and management goals across a wide range of taxonomically similar species. For studies of odontocetes (N= 223), detailed extraction was limited to a maximum of 10 studies per species that were selected to include the range of primary methods used between the years 2000-2018 (N = 120 studies). Only four genera, *Tursiops, Delphinus, Physeter, Pontoporia* were represented by more than 12 studies. Of the studies available for these species, detailed extraction was completed for (a) the most recent and included (b) the range of study methods available. Variable definitions and schema codes for all levels of data extraction are described in (Table B2).

Table B2. Coding schema used in data extraction for All (N= 356 abstracts) and Odontocete studies (N = 120 full text) used in meta-analysis

Studies Included	Variable	Definition
All	Sub-order	Coded, Odontocete or Mysticete
All	Family	Coded, based on genus
All	Common Name	Common name reported in article
All	Genus	Latin
All	Species	Latin
All	Ocean Basin	Coded, all ocean basins in the study region
All	Size of Species Range	Coded, at the level of the species (not the population, and not the study?). Select: Small (river, isolated seas or

Studies Included	Variable	Definition
		geographic region), Medium (contiguous coastal, portion of an ocean basin), Large (whole or multiple ocean basin but latitudinal restriction), Global (most or all ocean basins)
All	Primary methods	Coded by method used on the majority of samples or was the focus of paper
All Odontocete	Conservation or management discussed?	Coded, yes/no. Key word search for "management" or "conservation" or "status" (of species) in abstract, or full text
Odontocete	Location	Specific location reported for the study area(s)
Odontocete	Jurisdiction	Coded, National jurisdiction(s) responsible for management of the study area. List all as reported or inferred from map
Odontocete	Habitat Type	Coded, Chose: Pelagic, Coastal, Both Pelagic & Coastal, River-Estuary, River-Estuary & Coastal
Odontocete	DU/MU/ ESU/ stock Definition?	Coded, yes/no, + copy of text & definition or citation if present. Do the authors use the terms: "designated unit", "Management unit", “Ecologically significant unit", "Evolutionary significant unit", “demographically independent population” or “designated "stock"?
Odontocete	MPAs?	Coded, yes/no. Key word search for "protected area" "sanctuary" “conservation area" "exclusion zone"
Odontocete	Other lines of evidence	What other methods were used to infer population structure?
Odontocete	Previous study?	Coded, yes/no. Do authors use data from a previous population structure study on the population?
Odontocete	Study goal	Copy paste from abstract/ introduction (for future keyword coding)
Odontocete	# Putative populations	# of putative populations described as the null hypothesis
Odontocete	Pop Boundaries based on	Coded, How were putative population boundaries determined? Select: (1) Sample locations, (2) Habitat, (3) Sightings surveys, (4) National jurisdiction, (5) Previous studies, (6) Last Glacial Maximum, (7) Latitude
Odontocete	Sample N (total)	#, final sample size for primary method as reported in results
Odontocete	Time Period	Year range data was collected, YYYY-YYYY
Odontocete	Map?	Coded, yes/no. Map of sample locations included in study?
Odontocete	Study area >(km)	Coded, distance between sample points in kilometres. Based on furthest distance between sample sites, as described or approximated using Google maps, select: >

Studies Included	Variable	Definition
		10/ 100/ 1,000/ 10,000/ 20,000 (kms)
Odontocete	Multi-scale?	Coded, yes/ no. Does the study compare samples within and between study areas?
Odontocete	Sites N	# sampling sites used in analyses (either pooled by strata or actual # if reported)
Odontocete	Samples/ site max-min	Range reported between smallest # and largest number of samples/site
Odontocete	Sample source	Coded, Source of data used in analyses, examples: biopsy, bycatch, museum collection, stranding, photos
Odontocete	Tissue Type	Coded, if relevant, type of tissue analysed: skin, blubber, bone, liver, muscle
Odontocete	Genetic markers	Coded, if relevant, select: (1) control region < 400bp, (2) control region < 500bp, (3) control region > 500bp, (4) cytochrome b, (5)mitogenome, (6) genome, (7) nuDNA, (8) microsats, (9)SNPs
Odontocete	Number of Microsatellites	# (final) of loci reported in results
Odontocete	Other methods used	Other methods or markers used, not already noted
Odontocete	SI used	Coded, Type of isotope analysis, select: d13C, d15N, d18O, bulk or CSIA
Odontocete	Structure detected?	Coded, yes/no. Assessment whether structure was detected in analysis (N >1 population = yes)
Odontocete	Pattern discussed?	Coded, yes/no + copy of text description. What is the structure or pattern described in the discussion?
Odontocete	Process discussed?	Coded, yes/no + copy of text description. What is the explanation or process described in the discussion?
Odontocete	Type of structure	Coded, What type of structure was detected? As mentioned or inferred. Scan of discussion + Keyword search, select best: (1) Panmixia, (2) Island- Isolated, (3) Geographic barrier, (4) IBD, (5)IBED, 6) Stepping Stone, (7) (Cline, (8) Metapopulation, (9) Breeding-Foraging, (10) Philopatry, (11) Eco-type, (12) Culture
Odontocete	Spatial pattern of population structure	Coded, Based on type of structure identified. (1) Panmixia, (2) Allopatry [Island-Isolated], (3) Parapatry [IBD, IBED, Stepping Stone, Cline, Metapopulation], (4) Sympatry [Philopatry, Ecotype, Culture]
Odontocete	# of populations	# of sub-populations determined in conclusion
Odontocete	Management Recommendations	Are any recommendations made for management of the population(s)? Copy paste recommendations from text for qualitative coding.

Data Review and Analysis

To understand the state of science for cetacean population structure, the data extracted for all (356) studies was summarized by taxon level (Committee on Taxonomy, 2021), species range size and ocean basin. Trends over time were assessed for the primary research method and the proportion of studies that acknowledged conservation and/or management within the title or abstract.

The detailed dataset for odontocetes was used to answer questions related to research design, assess patterns and drivers of population structure and summarize consideration for species conservation or management (Table B3). The effects of taxa-specific characteristics, habitat, and study methods on patterns of population structure were described and evaluated. Study goals were summarised, aggregated into themes and associated with relevant study methods, conservation or management objectives and ecological or evolutionary significance. The proportion of studies that recognized conservation or management applications was summarised, and for those studies that provided management recommendations, they were themed and qualitatively evaluated. The 2021 IUCN status for all species considered (or not) by studies in this review was summarized (IUCN, 2021). Finally, I documented, summarized and coded the range of population unit terms used by authors based on: (1) conservation vs. management objectives; (2) evolutionary vs. ecological concepts; and (3) inclusive versus exclusive significance to assess patterns in the level of population structure considered.

Table B3. Research questions and variables used in the exploratory review of odontocete population structure

Research Question	Variables Explored
Which cetacean species or families demonstrate population structure below the sub-species level?	Summarize studies with >1 population by species and family
Do inherent characteristics such as range size make population structure for a species more likely?	Summarize studies with >1 population by range size
Where has population structure been found? Is it unique to particular habitats or ocean basins?	Summarize studies with >1 population by habitat type and ocean basin

What are the patterns of population structure found in Cetacea?	Identify the range of patterns and processes described
How is population structure assessed and do experimental design or methods influence the results?	Describe trends in primary/ secondary methods over time. Identify range of spatial/temporal scales, sample size, sites.
What are the key goals or objectives of population structure studies?	Code main themes found across study objectives and summarize
How effectively have these studies contributed to conservation and management?	Compare IUCN status by distribution of studies. Summarize the % of publications that: mention management or conservation, define population unit make management recommendations, Evaluate the qualitative strength of management recommendations

S2. Supplementary Results

Based on the meta-analyses the following highlights additional results from the exploratory analyses of odontocetes relating to the influence of species-specific characteristics and experimental design on determinations of population structure.

The State of Cetacean Population Structure Science

There were 46 species and five subspecies of cetaceans considered by studies in this review, however the majority of all studies (N = 356) were focussed on the family Delphinidae (45%), predominantly *Tursiops sp.* (N= 72, 21%). Studies of Mysticetes were dominated by Humpback Whales (17%, N = 59). Iniidae, Neobalaenidae and Platanistidae families were not represented, while only 3 species of 23 currently recognized in Ziphiidae were considered. Multiple ocean basins were included in many studies, however the North Atlantic (N = 98) was most represented, followed closely by the south Pacific (N = 88) and north Pacific (N = 62) (Table B4). The 204 studies were coded for national jurisdiction of study area and analysed by OECD status (Figure B1; World Bank, 2021). There were 202 (65%) studies that acknowledged conservation and management within the abstract.

Table B4. Representation of ocean basins across all studies, including studies representing multiple basins (N = 39) and single basins (N= 317) so study N does not equal the total number of studies considered in this review. Percentages are calculated on the total study N.

Ocean Basin	Study N	Total %	Odontocete Study N	%
North Atlantic	98	27.5	34	28.3
South Pacific	88	24.7	27	22.5
North Pacific	62	17.4	13	10.8
South Atlantic	42	11.8	23	19.2
Arctic	23	6.5	8	6.7
Mediterranean Sea	20	5.6	16	13.3
Antarctic	12	3.4	1	0.8
Indian Ocean	12	3.4	4	3.3
Gulf of California	6	1.7	2	1.7
Gulf of Mexico	6	1.7	6	5.0
Arabian Sea	4	1.1	na	na
Black Sea	4	1.1	3	2.5
South China Sea	4	1.1	4	3.3
Caribbean	2	0.6	na	na
North Sea	2	0.6	2	1.7

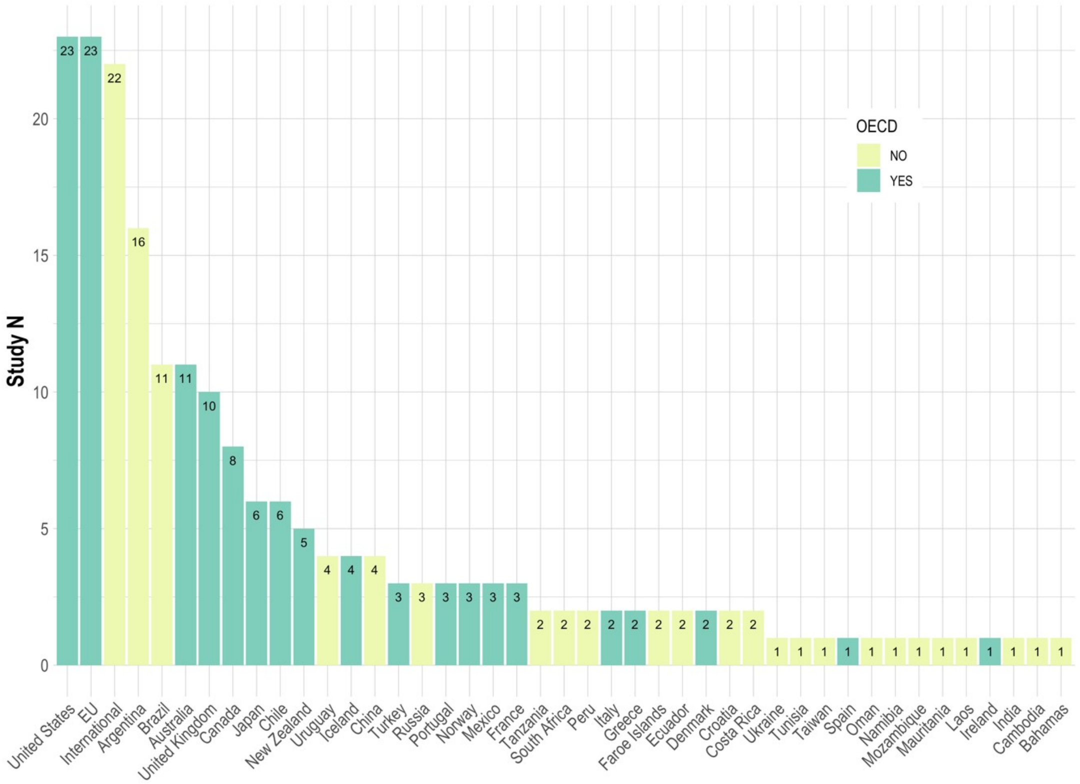


Figure B1. The distribution of study areas by national jurisdiction, where it was possible to determine from the abstract (N = 204). Countries are coloured by membership in the Organization for Economic Co-operation and Development (OECD). EU = European union, where study took place within the jurisdiction of multiple EU countries. Studies were considered "International" when they were global or occurred in more than one jurisdiction. Total number of studies within each jurisdiction is indicated on each bar.

Population Structure in Odontocetes

The detailed review of odontocetes included studies representing cetaceans from 23 separate genera and 37 different species. For cetaceans where structure was not detected, all but one genus (*Mesoplodon*, N = 1 study) were also represented by studies where population structure did occur. On average, structure appears greater for species with global range sizes, however population structure at K = 1 occurred across all range size classes (Figure B2). When considering the influence of specific ocean ecosystems and potential evolutionary history on population structure, 24% of studies spanned multiple ocean basins making effects unclear; however, for those specific to a single ocean basin, the Gulf of Mexico (K = 4), Indian Ocean (K = 3.5) and the Arctic (K = 3.0) had above average population structure (Mean = 2.8). Structure (K >= 1) was detected for all studies in the Gulf of Mexico, Indian Ocean, Arctic, and South China Sea (Figure B3).

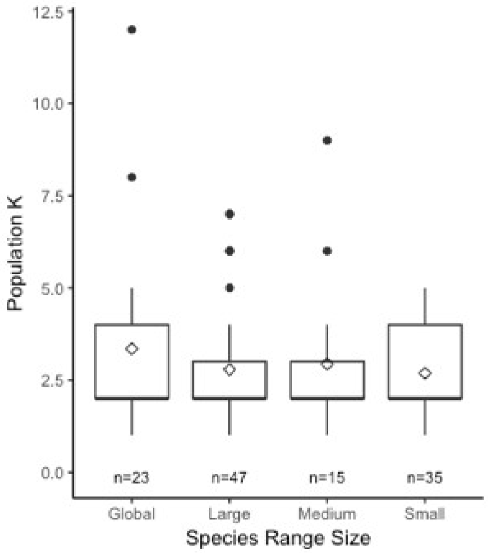


Figure B2. Population structure found in odontocete studies according to species range size classes. Small (river, isolated seas or geographic region), Medium (contiguous coastal, portion of an ocean basin), Large (whole or multiple ocean basin but latitudinal restriction), Global (most or all ocean basins).

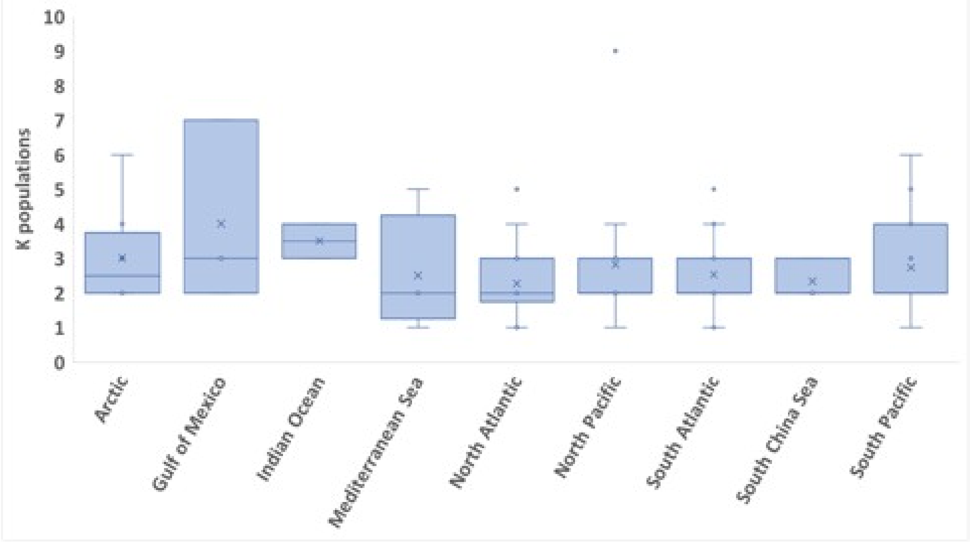


Figure B3. Population structure found across studies of individual ocean basins, median, interquartile range and outliers and mean (x) as indicated.

The iterative review process found ten different patterns and five processes of population structure described for cetaceans. Exploring patterns of population structure was complicated by pattern concepts that were also processes (e.g. Culture), as well as by studies that: (a) interpreted process(es) rather than patterns, (b) suggested multiple patterns / processes, or (c) did not have clear conclusions regarding either pattern or process. The summary is based on the dominant pattern or process discussed by the author, recognizing that further consideration may help parse patterns and processes as separate variables. Aside from panmixia, allopatric patterns had the smallest average K, but a substantial overlap with patterns in parapatry or sympatry (Figure B4).

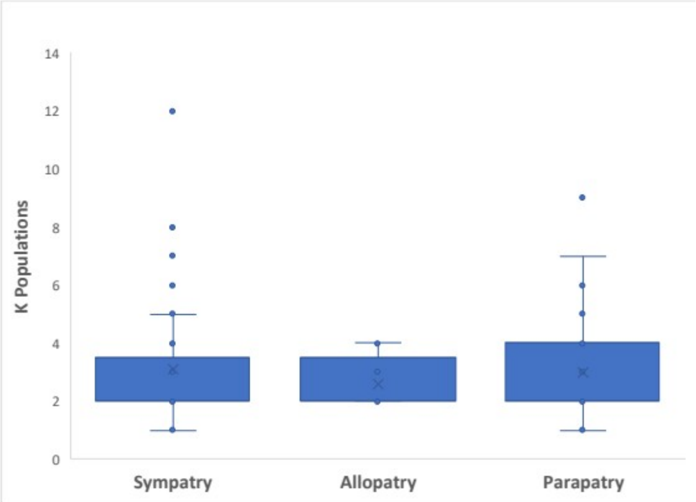


Figure B4. The number of k populations found in studies by spatial domain of population structure: Sympatry, Allopatry, or Parapatry. See Table 2.4 for details on specific patterns or processes.

Some aspects of experimental design appeared to influence the magnitude of population structure reported. The temporal scale of sampling was reported in 75% of studies and ranged between 1 - 147 years (mean = 16.7 yrs, median = 13 yrs), with the majority of samples collected beginning in 1990. There is a small positive trend in number of populations detected (K) with longer periods of sample collection, however there is no obvious break point as the range of population structure is found across all temporal scales (Figure B5). There was a lot of variation, but it appeared there was a weak positive relationship between K (number of populations) and the total sample size for tissue-based analyses Figure B6. Sampling design was largely opportunistic rather than random, and more than a third (37%) of the experimental strata are described as being defined by sample locations or data clusters. Because of the low number of studies where population structure was not detected, and the overall prevalence of combining genetic markers, there were no clear patterns between the different methods used for investigation and structure detected.

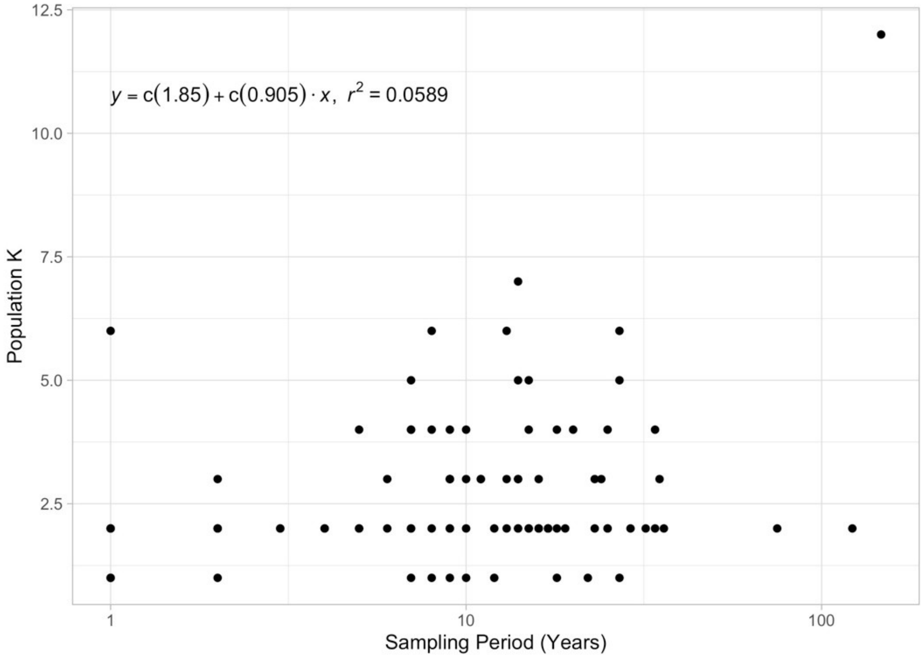


Figure B5. The number of k populations and temporal scale of sampling, where sample collection period was reported (74% of odontocete studies, N = 89)

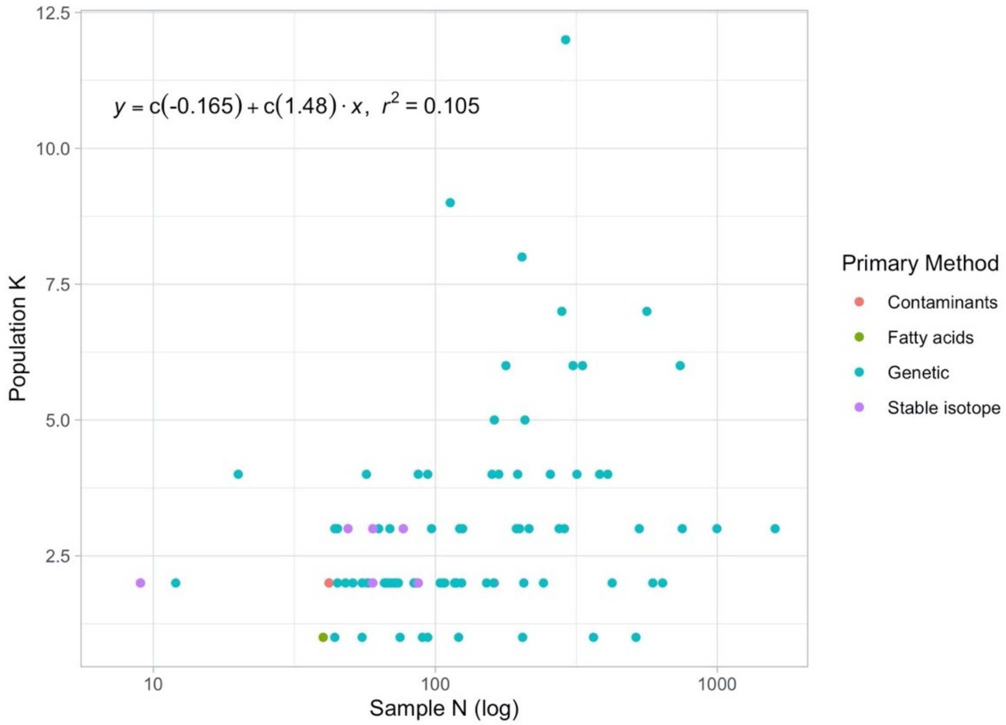


Figure B6. The number of k populations determined by studies in relation to the number of samples included in tissue-based methods (Genetics, Stable Isotopes, Morphometrics, Fatty Acids, Contaminants)

Consideration for Cetacean Conservation and Management

Out of 356 studies only 55% (N = 195) used one or more "Unit" terms (ESU, DIP, MU, Stock, etc) in the abstract of their study. There were 13 different classes of study goals identified (Table B5). Most studies (81%) had multiple goals, and the principal goals were to understand boundaries (85%), migration rates, connectivity or gene flow (30%) and characterize diversity (29%). Supporting conservation and management was mentioned as a study goal in just over 20% of studies.

In the full text analysis of odontocete studies, conservation and management was acknowledged by 84% of studies, specifically this was mentioned as a study goal by 21% of studies, consideration for marine protected areas (10%) and formalized by a management recommendation (68%). The relevance of conservation or management objectives and evolutionary or ecological significance to study goals that were identified is summarised in Table B5.

Table B5. Study goals and their applicable methods considered in light of relevant conservation or management objectives and evolutionary or ecological significance. Based on review of odontocete studies, number of studies identified and overall %. Studies could have one or more goals.

Study Goal	Study N (Total %)	Methods	Objective	Significance
Understand boundary	101 (85%)	Acoustic, Contaminants, Fatty acids, Genetic, Morphometrics, Parasites, Photo-identification, Stable isotopes, Tagging, Demography, Distribution, Phenotype, Habitat	Management/ Conservation	Evolutionary or Ecological
Understand migration rates, connectivity or gene flow	36 (30%)	Genetics, Tagging, Photo Identification, Demography, Distribution, Phenotype, Social Structure, Habitat	Management/ Conservation	Evolutionary or Ecological
Characterize diversity	34 (29%)	Genetics, Acoustics, Morphometrics, Stable Isotopes, Phenotype	Conservation	Evolutionary
Understand environmental correlates	30 (25%)	Acoustic, Contaminants, Fatty acids, Genetics, Morphometrics, Parasites, Stable isotopes, Tagging, Habitat	Management	Ecological
Support management/ conservation	25 (21%)	All methods	Management/ Conservation	Evolutionary or Ecological
Understand ecological mechanisms	17 (14%)	Acoustics, Contaminants, Fatty acids, Genetics, Morphometrics, Parasites, Stable isotopes, Tagging, Social Structure, Habitat	Management	Ecological
Understand evolutionary mechanisms	16 (13%)	Genetics	Conservation	Evolutionary
Understand past structure	15 (13%)	Whaling data, Genetics, Habitat	Conservation	Evolutionary
Influence of sex-bias	14 (12%)	Genetics, Photo-identification, Tagging, Morphometrics, Demography, Distribution, Social Structure	Management	Evolutionary
Influence of demography	12 (10%)	Genetics, Photo-identification, Tagging, Morphometrics, Stable Isotopes, Stomach Contents, Demography, Distribution, Social Structure	Management	Ecological
Identify population bottlenecks	8 (7%)	Genetics, Whaling data, Demography, Distribution	Conservation	Evolutionary
Implications for breeding-reproduction	4 (3%)	Genetics, Whaling data, Photo-identification, Tagging, Stable Isotopes, Demography,	Management	Evolutionary or Ecological

Study Goal	**Study N (Total %)**	**Methods**	**Objective**	**Significance**
		Distribution, Social Structure, Habitat		
Ne	4 (3%)	Genetics	Conservation	Evolutionary

APPENDIX C Chapter 4

Table C1. Variable inclusion rationale and data sources

Variable	Study objective	Study Question	Data Source
$\delta^{15}N$	Weaning Age	Estimate trophic level of foraging, variation over time, end of weaning period	GG Hatch Lab
$\delta^{13}C$	Foraging Behaviour	Estimate baseline primary productivity between regional ecosystems and weaning related influences	GG Hatch Lab
Location	Weaning Age Foraging Behaviour	Identify population level or ecosystem differences in weaning or foraging behaviour	Lat/Long from whaling records
GLG - Year	Weaning Age	Identify fine scale differences over the first five years	Counts from tooth specimens
Sex	Weaning Age Foraging Behaviour	Identify sex bias in maternal provisioning and foraging behaviour	Genetic analysis
Individual	Weaning Age	Identify between and within individual variation	Whaling records

Table C2. Summary of cetacean studies reporting nursing duration or weaning age by species, method, average age at weaning, and sample type.

Common name	Genus species	Method	N	Weaning age mean months	Range months	Oldest weaned age	Sample Type	Year	Reference
10 Mysticete species: Blue, Fin, Sei, Minke, Humpback, Bowhead, Pygmy Right, Gray, Bryde	*B. musculus, B. physalus, B. borealis, B. brydei, B. acutorostrata, B.novaeangliae, B. mysticetus, C. marginata, E. robustus*	Cow-calf/ Stomach contents	n.r.	8	4-12		Hunted animals	1984	Lockyer 1984
Commerson's dolphin	*Cephalorhynchus c. commersonii*	δ^{13}C and δ^{15}N - bone collagen	220	6	6-12	n.r.	Stranded/ bycatch	2013	Riccialdelli et al., 2013
Beluga	*Delphinapterus leucas*	δ^{15}N of tooth dentin GLGs	25	30	2-3 years	4 yrs	Hunted animals	2015	Matthews & Ferguson 2015
Short-beaked common dolphins	*Delphinus delphis*	Teeth structure - accessory lines	117	18	1-2 years	n.r.	Wild population	2013	Luque et al., 2013
Common dolphins	*Delphinus delphis*	n.r.	n.r.	5.5	5-6	n.r.	Hunted animals	1984	Reported in Perrin and Reilly 1984
South Atlantic Right whales	*Eubalaena australis*	Behaviour	16	13	12-14	14 months	Wild population	1982	Taber & Thomas 1982
North Atlantic Right whales	*Eubalaena glacialis*	Behaviour	22	12		14 months	Wild population	2010	Hamilton & Cooper 2010
Short-finned pilot whale	*Globicephala macrorhynchus*	Stomach contents/ Cow-calf method	n.r.	36	3.5-5 years	13 years	Hunted animals	1984	Kasuya & Marsh 1984
Short-finned pilot whale	*Globicephala macrorhynchus*	Stomach contents/ Cow-calf method	31	9		22 months	Drive fishery	1962	Sergeant 1962
Long-finned Pilot whales	*Globicephala melas*	Stomach contents	n.r.	22			Hunted animals	1962	Sergeant 1962

Common name	Genus species	Method	N	Weaning age mean months	Range months	Oldest weaned age	Sample Type	Year	Reference
Rissos Dolphin	*Grampus griseus*	δ^{15}N of tooth dentin GLGs	22	17.4		2.13 years	Stranded/ bycatch	2017	Evacitas et al., 2017
Rissos Dolphin	*Grampus griseus*	Cow-calf method	16	24		3 years	Drive hunted	2012	Bloch et al., 2012
Rissos Dolphin	*Grampus griseus*	Cow-calf method	21	18	1-1.6 years	3 years	Drive hunted	2004	Amano & Miyazaki 2004
Northern bottlenose whale	*Hyperoodon ampullatus*	Stomach contents	n.r.	12		n.r.	Hunted animals	1979	Benjaminsen & Christensen 1979
Killer whale	*Orcinus orca*	δ^{15}N of tooth dentin GLGs	11	42	3- 4 years		Stranded animals	2009	Newsome et al., 2009
Killer whale	*Orcinus orca*	Behaviour	n.r.	18		n.r.	Captive born	1999	Dahlheim & Heyning 1999
Killer whale	*Orcinus orca*	Behaviour		>12			Wild	1982	International Whaling Commission 1982
Killer whale	*Orcinus orca*	Behaviour	n.r.	24		n.r.	Wild population	1979	Dahlheim & Heyning 1979
Dall's porpoise	*Phocoena dalli*	Stomach contents	n.r.	24	6-36	3 years	Hunted animals	1999	Ferrero & Walker 1999
Harbour porpoise	*Phocoena phocoena*	Teeth structure - accessory lines	167	12		3 years	Stranded animals	2009	Luque et al., 2009
Harbour porpoise	*Phocoena phocoena*	Behaviour	1	9	8-10		Live capture	2003	Lockyer & Kinze 2003
Sperm whale	*Physeter macrocephalus*	Stomach contents	27	7.5 years	2-13 years	13 yrs	Catch data	1984	Best et al., 1984
Franciscana	*Pontoporia blainvillei*	δ^{15}N of dorsal muscle tissue/ stomach contents	54	12		2 years	Bycatch	2017	Viola et al., 2017.

Common name	Genus species	Method	N	Weaning age mean months	Range months	Oldest weaned age	Sample Type	Year	Reference
Franciscana	*Pontoporia blainvillei*	$\delta^{15}N$ of whole tooth	125	48			Bycatch	2016	Troina et al., 2016
Franciscana	*Pontoporia blainvillei*	Stomach contents	26	7			Bycatch, calves	2013	Denuncio et al., 2013
Franciscana	*Pontoporia blainvillei*	Stomach contents	110	7			Bycatch	2002	Rodríguez et al., 2002
False killer whales	*Pseudorca crassidens*	$\delta^{13}C$ and $\delta^{15}N$ bone collagen		23	19-24		Stranded animals	2015	Riccialdelli & Goodall 2015
False killer whales	*Pseudorca crassidens*	Behaviour	2	24		2 years	Captive born	1999	Clark & Odell 1999
Guianna dolphin	*Sotalia guianensis*	Teeth structure - accessory lines	71	8	6.7 - 10.3		Bycatch	2003	Rosas et al., 2003
Pantropical spotted dolphin	*Stenella attenuata*	Stomach contents	203	9		2 years	Bycatch	2004	Archer & Robertson 2004
Pantropical spotted dolphin	*Stenella attenuata*	Cow-calf method	n.r.	20		60 months	Hunted animals	1984	Reported in Perrin and Reilly 1984
Striped dolphin	*Stenella coeruleoalba*	Cow-calf method	n.r.	16		36 months	Hunted animals	1984	Reported in Perrin and Reilly 1984
Striped dolphin	*Stenella coeruleoalba*	Cow-calf method/ Stomach contents	45	18	3 yrs	n.r.	Drive fishery	1977	Miyazaki 1977
Spinner dolphin	*Stenella longirostris*	Cow-calf method	n.r.	21		34 months	Hunted animals	1984	Reported in Perrin and Reilly 1984
Indo-pacific bottlenose dolphins	*Tursiops aduncus*	Behaviour	22	42		6 yrs	Wild population	2004	Kogi et al., 2004.
Bottlenose dolphins	*Tursiops sp.*	Behaviour	74	48		8 years	Wild population	2000	Mann et al., 2000

Common name	Genus species	Method	N	Weaning age mean months	Range months	Oldest weaned age	Sample Type	Year	Reference
Bottlenose dolphins	*Tursiops sp.*	Behaviour	summary	19	18-20	38 months	Wild and captive	1984	Reported in Perrin & Reilly 1984
Bottlenose dolphins	*Tursiops truncatus*	Behaviour	11	36		4 yrs	Wild population	2018	Baker et al., 2018
Bottlenose dolphins	*Tursiops truncatus*	Behaviour	136	47		8.59 yrs	Wild population	2018	Karniski et al., 2018
Bottlenose dolphin	*Tursiops truncatus*	δ15N - tooth dentin age classes	15	36	2-4 years		Stranded animals (female)	2015	Rossman et al., 2015
Bottlenose dolphins	*Tursiops truncatus*	δ13C and δ15N - whole teeth	49	24		n.r.	Stranded animals (age 1-5)	2015	Fruet et al., 2015
Bottlenose dolphins	*Tursiops truncatus*	δ 13C and δ 15N - teeth and skin samples	60/32	24		3 years	Stranded animals	2008	Knoff et al., 2008
Bottlenose dolphins	*Tursiops truncatus*	n.r.	n.r.	12		7 years	Wild population	1999	Wells et al., 1999
Bottlenose dolphins	*Tursiops truncatus*	Stomach contents	n.r.	12		3 years	Stranded/ bycatch	1990	Cockcroft & Ross 1990

APPENDIX D Chapter 5

Table D1. Sample size of the dorsal fin photograph identification catalogue for the Scotian Shelf northern bottlenose whales.

Study year	Photography method	# of IDS Left	Right	# of Photographs Left	Right
1988	Film	6	5	13	14
1989	Film	67	68	359	276
1990	Film	127	118	813	827
1991	Film	2	-	2	-
1993	Film	38	39	160	128
1994	Film	36	29	105	81
1995	Film	7	12	11	34
1996	Film	74	75	587	504
1997	Film	85	82	533	434
1998	Film	68	65	892	780
1999	Film	56	55	506	464
2001	Film	43	44	141	154
2002	Digital/Film	87	84	515	416
2003	Digital/ Film	24	24	81	87
2006	Film	29	23	160	125
2007	Digital	9	12	28	27
2008	Digital	5	4	9	4
2009	Digital	2	-	2	-
2010	Digital	51	52	600	531
2011	Digital	114	115	1339	1170
2013	Digital	47	41	561	419
2015	Digital	86	83	1702	1565
2016	Digital	93	95	4788	4392
2017	Digital	18	18	343	347
2019	Digital	71	73	1277	1215

Table D2. Overall proportion of marked individuals with sex classification based on molecular sex (Male XY and Female XX) and the catalogue's system of sex-age classification which supplements molecular sex identifications based on melon photographs (Male-mature male and Female-juvenile).

Mark **(N)**	**Male XY** **(41)**	**Female XX** **(46)**	**Male-mature male** **(180)**	**Female – juvenile** **(421)**
Notch	0.80	0.52	0.65	0.34
Indent	0.29	0.13	0.12	0.05
Entanglement	0.20	0.07	0.12	0.04
Propeller	0.07	0.00	0.05	0.00
Large fin scar	0.34	0.07	0.20	0.08
Patch	0.29	0.37	0.19	0.24

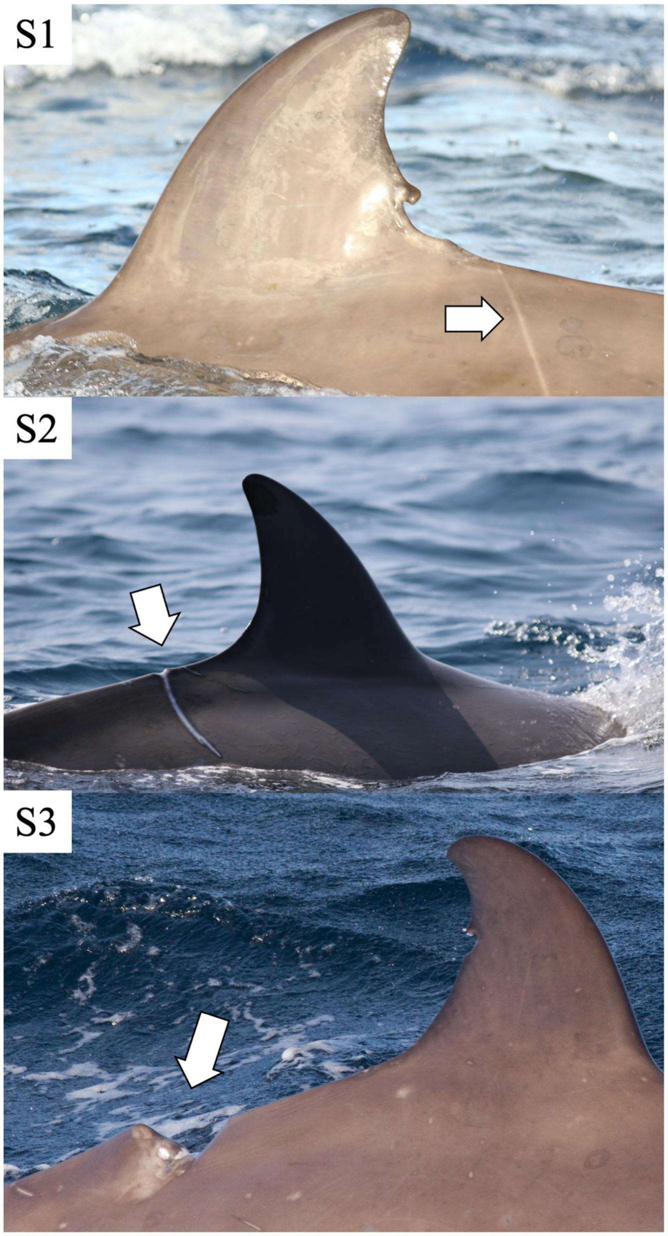


Figure D1 Anthropogenic scar severity scale: S1 is the least severe scar included in the classification, S2 is assessed as a moderately severe injury, likely permanent and S3 is a severe permanent injury. Example provided for S3 could be attributed to either a propeller-vessel strike or entanglement injury.

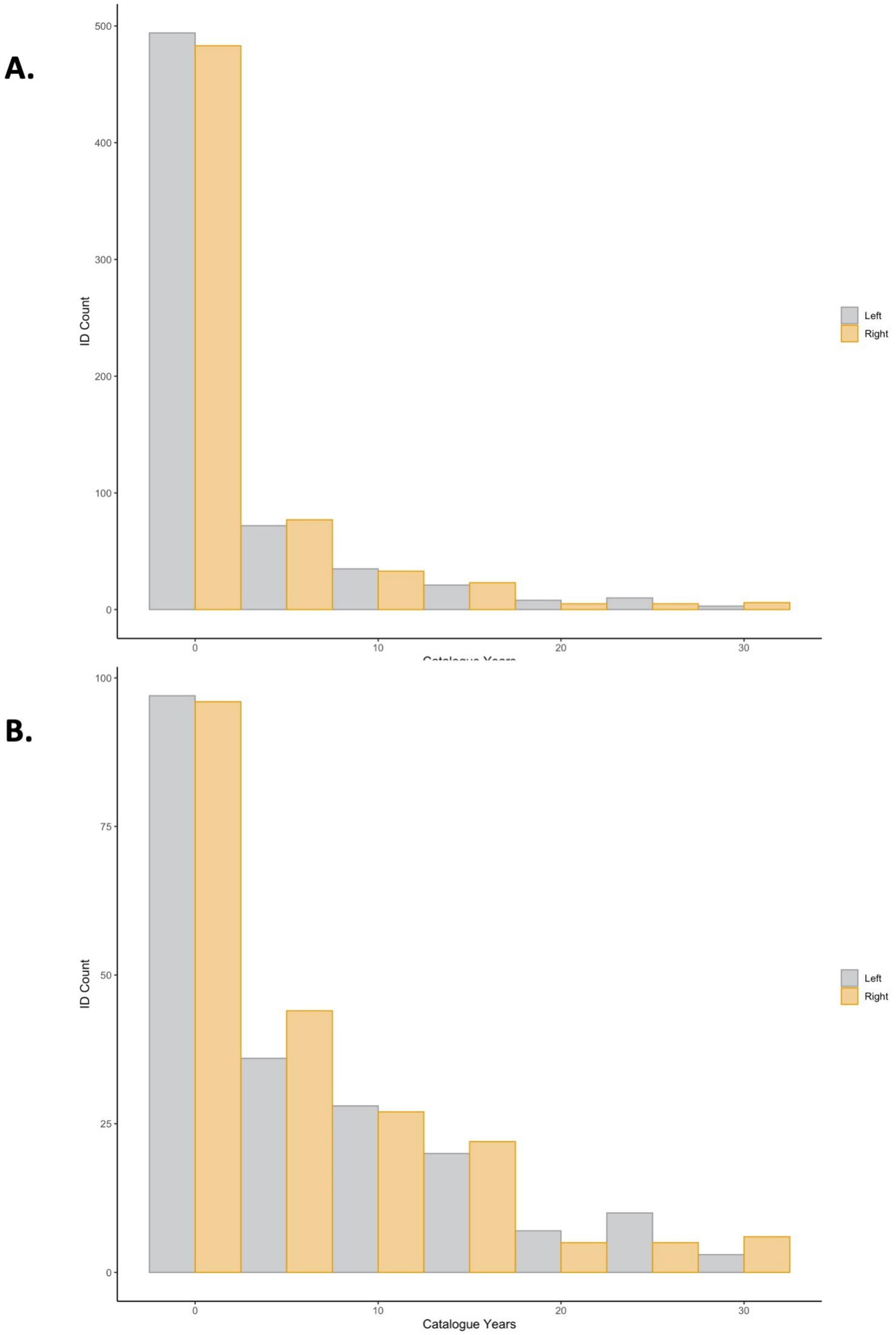

A.
500
400
300
200
100
0
ID Count
0
10
20
30
Catalogue Years
Left
Right
B.
100
75
50
25
0
ID Count
0
10
20
30
Catalogue Years
Left
Right

Figure D2. Histograms of catalogue years, the number of years with photographs for an ID, for (A) all IDs and (B) reliable IDs only, 1988-2019. Excludes single year IDs first seen in 2019. Gray and gold bars are Left and Right sides respectively.

APPENDIX E Chapter 6

Supplementary Methods and Materials

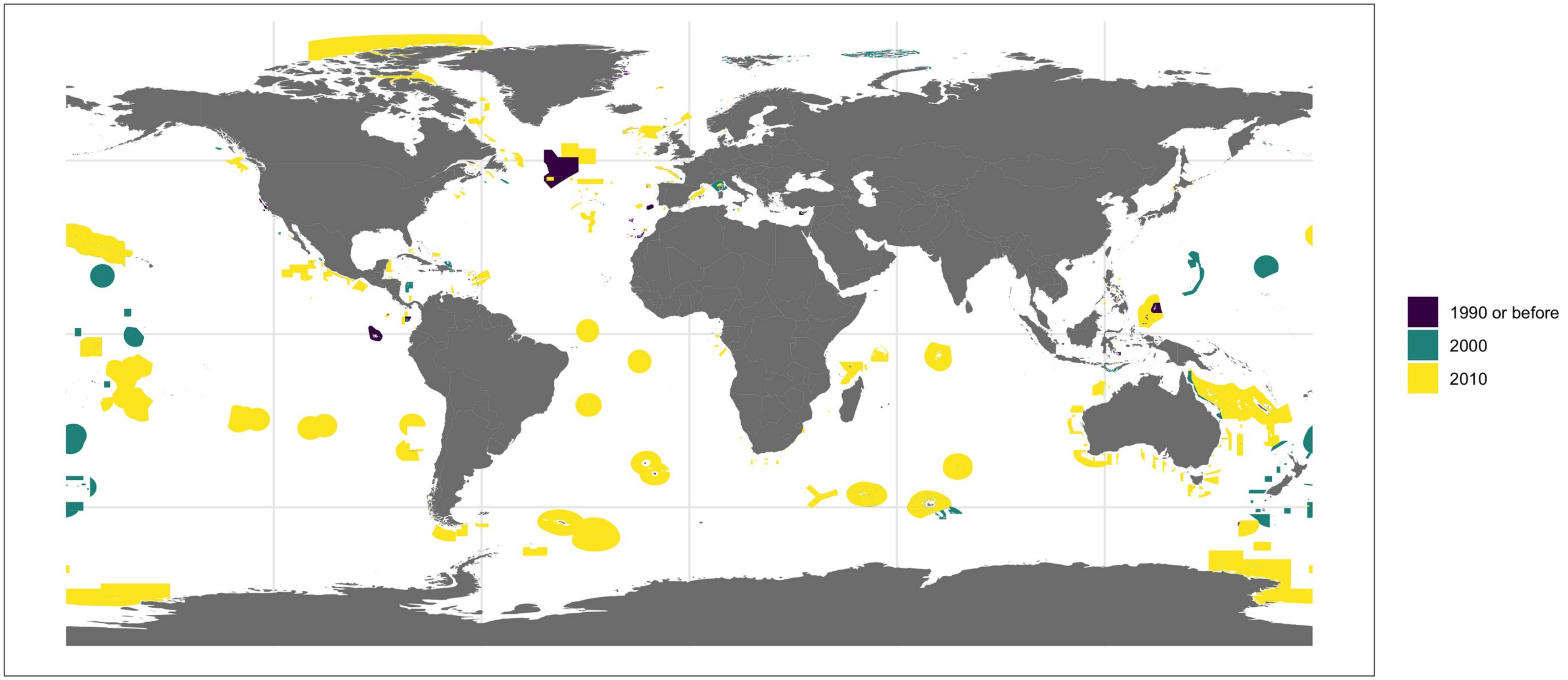


Figure E1. Global distribution of pelagic areas (>200m depth) protected by MPAs. Coloured by decade implemented: 2010-present (yellow), 2000-2010 (teal), prior to 2000 (purple). Source: World database on protected areas (2021)

S1. Supplementary Methods:

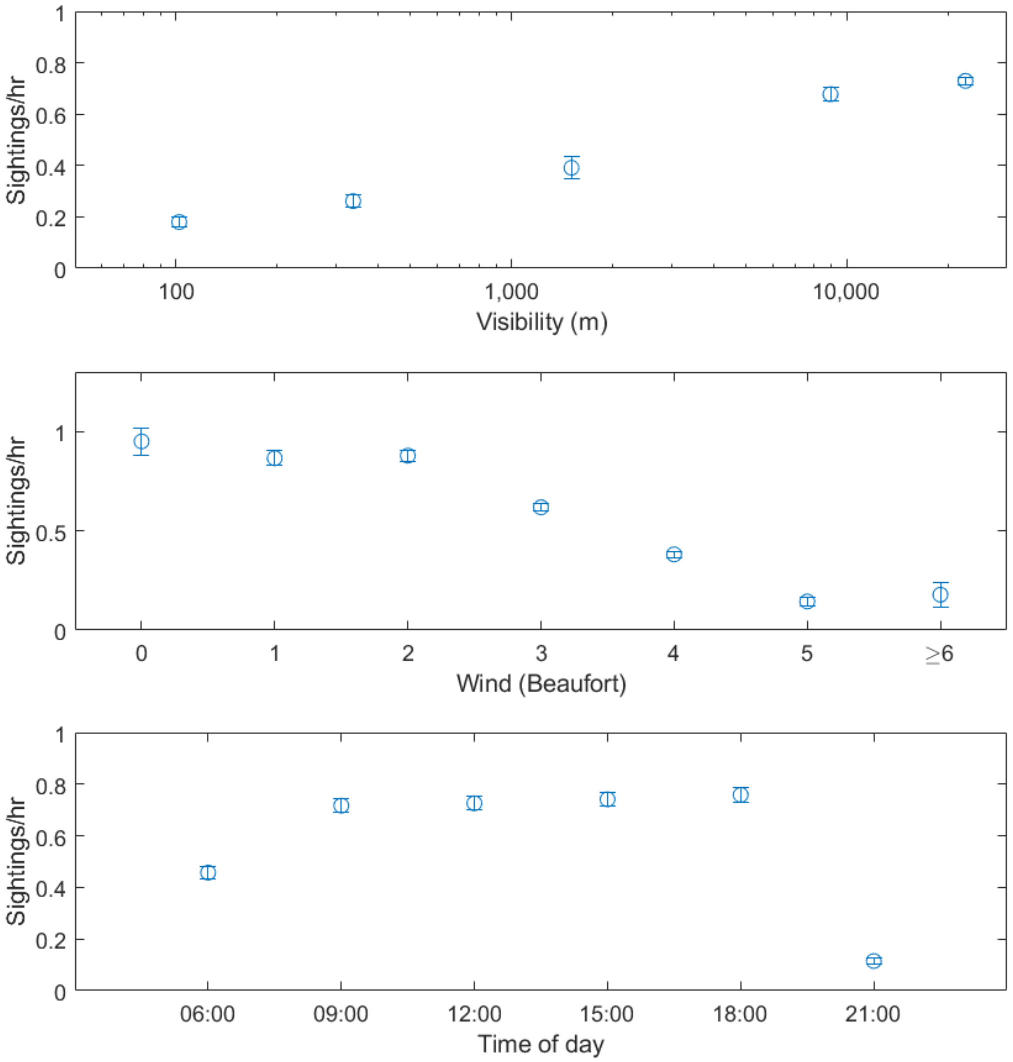


Figure E2. Sighting rates in different environmental conditions. Standard errors were estimated using the Poisson approximation.

Additional Analyses of Sighting Rates

In addition to the methodology described in the main text, we analyzed the sighting rate data in several other ways (Table E1): without aggregating 3-hr intervals by year-month-canyon and using environmental measures as covariates (this left substantial autocorrelation, *r*~0.3, in the residuals); without aggregating 3-hr intervals by year-month-canyon, using environmental measures as covariates and an intercept varying among days (this had poor convergence of the MCMC chains); aggregating by year-month-canyon but using a fixed intercept (this had substantial overdispersion, variance inflation factor ~4.0); aggregating and using a gamma-Poisson rather than Poisson model to model overdispersion (this had poor convergence of the MCMC chains). Despite the issues with these analyses, parameter estimates (β_1, β_2, T, etc.) were similar from all approaches.

Table E1. Bayesian regression models fit to sighting data including canyon (but not month) effects. In some cases, corresponding generalized linear models (GLMs) were also fit to check for autocorrelation of residuals (indicating lack of independence) and dispersion (indicating fit of Poisson model).

				Parameter estimates:			
Distribution	**Data units**	**Effort**	**Intercept**	**β1**	**β2**	**T**	**Comments**
Poisson	Sightings in each 3-hr period	Environmental records as covariates	Constant	-0.036	0.048	2010	Autocorrelation of residuals (r=0.3) in GLM version
Gamma-Poisson (negative binomial)	Sightings in each 3-hr period	Environmental records as covariates	Constant	-0.037	0.048	2010	Poor convergence ($\hat{R}$>3.0)
Poisson	Sightings in each 3-hr period	Environmental records as covariates	Varying by day	-0.023	0.051	2010	Poor convergence ($\hat{R}$>1.5)
Poisson	Sightings aggregated by year-month-canyon	Index of effort aggregated by year-month-canyon	Constant	-0.036	0.048	2011	Dispersion 5.8 in GLM version
Poisson	Sightings aggregated by year-month-canyon	Index of effort aggregated by year-month-canyon	Varying by year-month canyon	-0.037	0.048	2010	Good
Gamma-Poisson (negative binomial)	Sightings aggregated by year-month-canyon	Index of effort aggregated by year-month-canyon	Constant	-0.023	0.044	2009	Poor convergence ($\hat{R}$>1.3)

Table E2. Fits of variable-intercept Poisson Bayesian models to year-month-canyon aggregated sighting rates, together with parameter estimates.

			Parameter estimates:		
Model	**WAIC**	**SE (WAIC)**	**Initial trend (β_1/yr)**	**Final trend (β_2/yr)**	**Canyon effect (μ)**
Piecewise year, Month, Canyon	476.6	52.7	-0.036	0.048	-0.340
Piecewise year, Canyon	467.4	51.1	-0.037	0.048	-0.342
Piecewise year (2004), Month, Canyon	515.7	60.5	-0.043	0.047	-0.313
Piecewise year (2004), Canyon	508.2	58.6	-0.044	0.047	-0.311
Linear year, Month, Canyon	535.4	65.2	-	-	-0.361
Month, Canyon	535.2	66.1	-	-	-0.306
Linear year, Canyon	529.4	63.8	-	-	-0.363
Canyon	528.5	65.3	-	-	-0.310
Piecewise year, Month	529.4	61.1	-0.044	0.045	-
Piecewise year	524.6	60.7	-0.043	0.042	-
Piecewise year (2004), Month	516.2	59.2	-0.050	0.047	-
Piecewise year (2004)	508.9	57.0	-0.052	0.047	-
Linear year, Month	538.3	61.8	-	-	-
Month	533.3	61.8	-	-	-
Linear year	533.5	60.3	-	-	-
Constant	526.6	60.0	-	-	-

Table E3. Results of mark-recapture analyses, including heterogeneity of identification using mixture models, of population trends for Scotian Shelf northern bottlenose whales, together with parameter estimates.

	Left-fins			Right-fins		
Model	**AIC**	**Initial trend (β1/yr)**	**Final trend (β2/yr)**	**AIC**	**Initial trend (β1/yr)**	**Final trend (β2/yr)**
Constant	1439.3	-	-	1406.4	-	-
Linear year	1441.2	-	-	1406.2	-	-
Piecewise year	1439.5	-0.008	0.043	1405.9	-0.017	0.030
Piecewise year (2004)	1439.5	-0.021	0.022	1403.9	-0.017	0.030

Evaluating Cumulative Human Impacts

As a starting point we reviewed the available literature and government recovery plans (COSEWIC, 2011; Fisheries and Oceans Canada & Department of Fisheries and Oceans, 2016; Harris et al., 2013; Whitehead et al., 2021) to identify the list of stressors or threats as well as all the conservation measures in place for northern bottlenose whales. We limited our analyses of human impacts to data representative of the intensity of stressors with publicly available spatial and temporal information that covered the area and period of interest. Specific threats that were identified but could not be included due to a lack of spatial data were estimates of pollution, namely plastic debris, contaminants and oil spills. However, other stressors that were included could also be considered proxies for these threats (e.g., plastic and debris from fishing vessel activities, oil spills and contaminants associated with wells and other exploratory oil and gas activities or shipping). We followed the process outlined by Halpern et al. (2015) as a framework to model cumulative human impacts on marine ecosystems: (1) we assembled the temporal and spatial data on the intensity of the different activities associated with the stressors that covered the study area, which was defined by a 50km buffer along the 1000m isobath as an estimate of the region influencing NBW and their habitat; (2) we normalized the intensity data so that it scaled from 0-1, where "effort" information was available over time (year) or space (1km^2 grid cell), and presence-absence data was treated as binary (0/1); (3) we weighted each layer by an assessment of the sensitivity of NBWs to the stressor. Weights were evaluated based on the spatial extent, temporal frequency and impact on NBW (direct or indirect) and quantified using a three-point scale (Table E4); (4) we multiplied each layer by the NBW sensitivity weight and summed the cumulative weighted effort of stressors in each period defined by the mid-point of the timeline as well as the creation of the Gully MPA, before and after 2004. This resulted in a raster of CHI scores for 1988-2004 and for 2005-2019 for the study area. CHI was then used to characterize the relative difference in impacts across the study, in protected areas and NBW critical habitat, which could be compared with trends in NBW population size over this period.

Table E4. Contribution of each stressor to cumulative CHI for the periods (a) 1988-2004 and (b) 2005-2019.

(a) **Early period 1988-2004**	**Median impact**	**Max impact**	**% CHI**
Military Activities	0.13	0.13	0.02
Shipping Traffic	1.70	3.00	0.39
SST Anomalies	0.77	1.00	0.18
Fishing Effort	1.22	7.10	0.37
OG Exploration	0.46	1.25	0.05
OG Operations	2.00	6.00	0.00
(b) **Contemporary period 2005-2019**			
Military Activities	0.15	0.15	0.02
Shipping Traffic	1.70	3.00	0.39
SST Anomalies	0.81	1.00	0.19
Fishing Effort	1.16	7.04	0.37
OG Exploration	0.25	0.25	0.02
OG Operations	2.00	6.00	0.00

Description of Each Stressor and Data Sources

Oil and Gas Exploration and Development Activities – Publicly available spatial and temporal data was collated from the Canada-Nova Scotia Offshore Petroleum Board (C-NSOPB) and the Canada-Newfoundland and Labrador Offshore Petroleum Board (C-NLOPB) websites (CNLOPB, 2021; CNSOPB, 2021). Shapefiles for existing pipelines, wells, platforms, seismic surveys (2D/3D), exploratory licenses, significant discovery licenses, production licenses and sector announcements provided as part of the call for bids processes were downloaded, cleaned, and checked for duplication. Activities occurring before and after 2004 were identified based on metadata in the shapefile or provided elsewhere on the website. Spatial data for individual seismic surveys that occurred after 2004 were not publicly available, however according to the NSOPB (CNSOPB, 2021) there have been few seismic surveys in the Scotian Shelf portion of the study area in the contemporary period. Seismic surveys would largely occur in the areas of exploratory licenses issued during this period, which were used as a proxy for seismic survey effort. Pipelines, wells and platforms were treated as presence absence data as per Halpern et al. (2008), and while some of this infrastructure has been decommissioned since it was built, the ongoing risk associated with pollution continues over the entire study period. The extent of oil and gas development activities in the portion of the study area regulated by the C-NLOPB may be incomplete, however much of the data on seismic survey effort that occurred in the early period was available.

Fishing effort – Two data sources were used to assess fishing effort due to gaps in coverage for different gear types between the two: (1) Halpern et al.'s (2015) global analysis of fishing effort based on reported catch landings, which were summarized by “destructiveness” of different gear types; and (2) Butler et al.'s (2019) regional analysis of fishing effort which summarises fishing effort by soak time or vessel hours compiled by gear type. The specific Halpern et al. (2015) fishing effort layers included demersal destructive (e.g., bottom trawl), demersal non-destructive high bycatch (e.g., pots, traps), demersal non-destructive low bycatch (e.g., hook and line), and pelagic low bycatch (e.g., hook and line). Halpern et al. (2015) provided data for two time periods, the earlier period was based on 2006 fishing effort reports and used here to represent the early period, the later period was based on 2011 and was used to represent the contemporary period. Halpern et al.'s (2015) global resolution dataset did not record effort for pelagic high bycatch fisheries (longline) in the study area, but as longline fisheries do occur in the region and have a record of interactions with NBW, we used layers produced Butler et al. (2019) to capture the intensity and distribution of effort of these fisheries. Butler et al.'s (2019) analysis covered fishing effort from 2002-2017 across the majority of the extent of the study area, with the exception of the eastern Grand Banks portion of Newfoundland. The average of annual effort was used without modification for longline fisheries in the early period. For fishing effort in the contemporary period Zone one of the

Gully MPA was removed from all fishing effort layers as the aggregated time periods and resolution of the underlying data sets would not accurately capture the reduction in fishing effort across this small area.

Shipping – From Halpern et al. (2015), methods described in detail in the original publication however briefly, this layer combines several years of shipping data but is mostly a static description of shipping intensity and distribution during 2011. Intensity of global shipping traffic may have increased, but spatially is likely to remain relatively stable across the study area. As historical or contemporary data on this stressor is not easily accessible, and restrictions on ships transiting within the area of Zone one are voluntary, the same layer is used for both periods.

Climate change — Based on a proxy of sea surface temperature (SST) anomalies, from Halpern et al. (2015) methods described in detail in the original publication however briefly, this layer is based on Advanced Very High Resolution Radiometer (AVHRR) SST data. Halpern et al. (2015) defined anomalies as exceeding the average variation in regional weekly climatology, and established those occurring in the baseline period 1985-1990. The intensity of SST anomalies represents the difference in the frequency of anomalies for 2000-2005 (used for the early period) and the 2005-2010 (used for contemporary period) from the baseline SST in 1985-1990.

Military exercises– The Department of National Defence has designated Firing Practice and Exercise Areas off the coasts of Canada. Activities in these areas may include bombing practice from aircraft, air-to-air, air-to-sea or ground firing, and anti-aircraft firing, etc. In Atlantic Canada, this includes sea area employments for sub-surface operations and firing exercises (FIREX) (Department of National Defense Government of Canada, 2021). Polygon shapefiles of the designated areas were downloaded from the Canadian Government open data portal (Department of National Defense Government of Canada, 2021). With no effort data on military exercises in these areas across the entire period, we interpreted them to represent threat potential for military exercises. Although noise impacts can extend outside these areas, the most acute threat would occur inside the exercise areas. According to the Canadian Coast Guard Navigational Warnings (NAVWARNs), previously known as Notices to Shipping (NOTSHIPS), there were 44 notices of military exercises in areas overlapping with beaked whale habitat, on 54 days in 2019 (the first year notices were made available online), lasting between 1-5 days each (Government of Canada, 2021). However, this included 8 (non-consecutive) days of the biannual international CUTLASS FURY exercises, which began in 2016 and have been described as the "largest international military exercise in Canadian waters" (The Canadian Press, 2016). Excluding CUTLASS FURY 2019, we used the proportion of days per year with notifications to mariners (46 days = 12.6%) as a baseline for effort across the area in any given year. Although there is little information on the extent of military activity in the early period, in the contemporary period there have been two large international exercises as part of the public record in 2016 and 2019. To account for the impact of the international exercises, we have increased the % effort to 15% per year in the period after 2004. In addition, we removed the MPA from this layer in the contemporary period, as the MPA management plan (Fisheries and Oceans Canada, 2017) indicates that although the Gully MPA cannot legally exclude military activities, in practise the government agrees that such exercises do not occur inside the MPA.

Table E5. Difference in absolute CHI scores between 2005-2019 and 1988-2004 over all grid cells in the 228,842 km^2 study area and within conservation areas. More extreme negative values indicate a reduction in stressor intensity in the area.

Region	**Median**	**Maximum**	**SD**	**Area**
Study area	-0.086	3.471	0.369	228,842 km^2
All conservation areas	-0.005	0.924	0.950	36,396 km^2
Critical Habitat for NBW	-0.664	0.045	0.950	988 km^2
Gully MPA - All Zones	-0.140	0.084	0.830	2364 km^2
Gully MPA - Zone one	-1.882	-0.527	0.718	477 km^2

Table E6. Area (km^2) of CHI increase and decrease over the period 1988-2019. Estimated based on the absolute difference from 0 in CHI scores and where increase or decrease was greater than the RMSE of difference in CHI between periods. RMSE of CHI between periods was = 0.398.

Difference threshold	**Area of CHI increase (km^2)**	**Area of CHI decrease (km^2)**
Absolute	76,300	154,000
>1 RMSE	11,700	54,700
>2 RMSE	678	10,300